Identification of *Hordeum spontaneum* Genotypes Resistant to Net Blotch Disease

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ABSTRACT

Wild barley (*Hordeum spontaneum*) is a progenitor of cultivated barley and naturally grows in Turkey. *H. spontaneum* genotypes possess superior characteristics for biotic and abiotic stress tolerance factors. In this study, 3 virulent *Pyrenophora teres f. maculata* and 3 virulent *P. teres f. teres* isolates were tested under greenhouse conditions in order to find net blotch resistant *H. spontaneum* genotypes. A total of 104 *H. spontaneum* genotypes were used. Twenty-six *H. spontaneum* genotypes which corresponded to 25% of the genotypes (genotypes numbered 8, 13, 14, 16, 22, 24, 27, 31, 37, 44, 47, 54, 58, 62, 65, 66, 69, 74, 78, 81, 89, 94, 99, 102, 104 and 107) exhibited reactions classified in the resistant group to 3 virulent *P. teres f. maculata* isolates. Eight *H. spontaneum* genotypes which corresponded to 7.6% of the genotypes (genotypes numbered 24, 27, 29, 33, 44, 54, 89 and 94) exhibited reactions classified in the resistant group to 3 virulent *P. teres f. teres* isolates. Six *H. spontaneum* genotypes which corresponded to 5.7% of the genotypes (genotypes numbered 24, 27, 44, 54, 89 and 94) exhibited reactions in the resistant group to both 6 virulent *P. teres f. teres* and *P. teres f. maculata* isolates. In addition, a considerable number of genotypes exhibited resistant group reactions to one or two isolates of both forms of the pathogen. These genotypes could be used for developing net blotch resistant barley cultivars.

Keywords: Barley; Disease resistance; *Hordeum spontaneum*; Net blotch; *Pyrenophora teres*

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1. Introduction

Wild barley (*Hordeum spontaneum*, syn: *Hordeum vulgare* subsp. *spontaneum*) is a progenitor of cultivated barley and naturally grows in Turkey (Kün 1996; Karakaya et al 2016). *Hordeum spontaneum* could hybridize with cultivated barley (*Hordeum vulgare*) and is an important plant for developing disease resistant barley cultivars. Wild barleys and barley landraces possess superior characteristics for abiotic and biotic stress tolerance factors (Ceccarelli & Grando 2000; Karakaya et al 2016; Çelik & Karakaya 2017). Because of these characteristics, it is advised to preserve *H. spontaneum* genotypes *in situ* and *ex situ* conditions for future research (Nevo 2012).

China, India, Central Asia, Near East, Mediterranean region, Ethiopia, Southern Mexico and Central and South America are eight main
regions in the world considered as plant gene centers. Turkey has a rich genetical diversity due to its location. Turkey is located at the intersection of the Mediterranean and the Near East gene centers, and it is on the historical migration and transportation routes of China, India, Central Asia and Ethiopia gene centers. In addition, Fertile Crescent region which includes Turkey’s Southeastern Anatolia region is known as the region where barley, wheat, lentil, hard-seeded fruit and olive are cultivated for the first time (Vavilov 1951). In addition, Jakob et al (2014) reported that Levant, Turkey and east of Turkey are three main regions of wild barley (H. spontaneum) populations. Wild barleys including H. spontaneum are commonly grown under natural conditions in Turkey (Karakaya et al 2016).

Net blotch is caused by the fungus Pyrenophora teres that belongs to ascomycota. Anamorphic stage of the fungus is named as Drechslera teres. Two biotypes of the fungus are recognized. Pyrenophora teres f. maculata incites the spot form and Pyrenophora teres f. teres incites the net form of the disease (Karakaya & Akyol 2006; Liu et al 2011). The disease is commonly reported from different parts of the world and reduces the yield and quality of barley considerably (Mathre 1982; Liu et al 2011; Karakaya et al 2014).

In this study, 3 virulent P. teres f. maculata (Ptm) and 3 virulent P. teres f. teres (Ptt) isolates were tested under greenhouse conditions in order to find net blotch resistant H. spontaneum genotypes. A total of 104 H. spontaneum genotypes were used. An abstract of this study has been published previously (Çelik Oğuz et al 2017).

2. Materials and Methods

2.1. Experimental materials

In this study, 107 wild barley (H. spontaneum) genotypes that collected from various parts of Turkey and conserved by Field Crops Central Research Institute (Ankara, Turkey) were used. The seeds of these H. spontaneum genotypes were multiplied from single heads. Out of 107 genotypes, 104 provided the sufficient amount of seeds and were included in this study. No sufficient seeds were obtained from genotypes No: 4, No: 15 and No: 41. Three Ptm isolates and 3 Ptt isolates that were found to be the most virulent ones in the study by Çelik Oğuz (2015) were used in determination of seedling stage resistance of 104 H. spontaneum genotypes under greenhouse conditions.

2.2. Treatments

Sterile mixtures of soil, sand and organic substances (60, 20, 20; v/v/v, respectively) were placed in plastic pots with diameters of 7 centimeters and depending on the quantity of seeds of genotypes, 5-10 seeds were placed to the pots. The pots were maintained under greenhouse conditions. Inoculation was performed at growth stages 12-13 (Zadoks et al 1974). The inoculum was prepared from cultures grown on Potato Dextrose Agar maintained at 16-23±2 °C night/day with a 10 h/14 h dark/light period. In order to prepare inoculum, mycelia were harvested from Petri dishes using a no.12 brush and concentration of inoculum was adjusted to 15-20×10⁴ mycelial parts/ml (Douiyssi et al 1998; Taşkoparan & Karakaya 2009; Usta et al 2014; Yazıcı et al 2015). One drop of Tween 20 was added to each 100 mL of inoculum (Aktaş 1995). Inoculum was sprayed onto barley seedlings using a hand sprayer and all leaves were covered with inoculum. The greenhouse temperature ranged between 18-23±1 °C night/day with a 10 h/14 h dark/light period. Plants were kept covered with nylon in transparent boxes with moist lids for 76 h following inoculation. Then, plants were maintained for another 48 h with the nylon uncovered and the ventilation of the boxes activated. There were three replications.

2.3. Evaluation of the disease

Plant evaluations were carried out seven days later following inoculation. For evaluation, scales developed for both forms of net blotch by Tekauz (1985) were used. Plant evaluations were based on lesion size, morphology, necrosis and chlorosis. Scale values of 1, 2 and 3 were considered as resistant group in this study. In the scale for the spot
form of net blotch, seven numerical classes were defined (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 of net blotch scale ten numerical classes were defined (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, and 10= VS: very susceptible). Resistant or moderately resistant genotypes have small net blotch lesions. Moderately susceptible or susceptible genotypes have chlorotic zones surrounding the necrotic areas and coalescence of these areas and death of leaves can occur.

2.4. Data analysis

Experiment was carried out using randomized block design with three replications. Data were square root transformed before statistical analysis. Separate two way analysis of variance was performed for each isolate and means of responses of *H. spontaneum* genotypes were separated by Least Significant Difference (LSD) test. Statistical tests were accomplished using JMP software (version 11; SAS Institute).

3. Results and Discussion

Seedling resistance reactions of 104 wild barley genotypes to 3 virulent *Ptm* isolates and 3 virulent *Ptt* isolates were determined. Analysis of variance revealed significant differences among the *H. spontaneum* genotypes (P<0.01). Response of the genotypes ranged between resistant (scale value 1) and susceptible (scale value 9) (Table 1). Thirty-nine, 9 and 2 genotypes exhibited moderately resistant, resistant-moderately resistant and resistant reactions to *Ptm* isolate 13-179 PTM, respectively. Fifty-three, 9 and 3 genotypes exhibited moderately resistant, resistant-moderately resistant and resistant reactions to *Ptm* isolate 13-167 PTM, respectively.

Twenty-six *H. spontaneum* genotypes which corresponded to 25% of the genotypes (genotypes numbered 8, 13, 14, 16, 22, 24, 27, 31, 37, 44, 47, 54, 58, 62, 65, 66, 69, 74, 78, 81, 89, 94, 99, 102, 104 and 107) exhibited reactions classified in the resistant group to 3 virulent *Ptm* isolates.

Nine and 1 genotypes showed moderately resistant and resistant reactions to *Ptt* isolate GPS 18 PTT, respectively. Thirteen, 6 and 2 genotypes exhibited moderately resistant, resistant-moderately resistant and resistant reactions to *Ptt* isolate UHK 77 PTT, respectively. Twenty-one and 8 genotypes exhibited moderately resistant and resistant-moderately resistant reactions to *Ptt* isolate 13-130 PTT, respectively.

Eight *H. spontaneum* genotypes which corresponded to 7.6% of the genotypes (genotypes numbered 24, 27, 29, 33, 44, 54, 89 and 94) exhibited reactions classified in the resistant group to 3 virulent *Ptt* isolates.

Six *H. spontaneum* genotypes which corresponded to 5.7% of the genotypes (genotypes numbered 24, 27, 44, 54, 89 and 94) exhibited reactions in the resistant to moderately resistant group range (Tekauz (1985) scale 1 to 3) to both 6 virulent *Ptt* and *Ptm* isolates. In addition, a considerable number of genotypes exhibited resistant to moderately resistant reactions to one or two isolates of both forms of the pathogen (Table 1).

Wild barleys are important resistance sources for controlling biotic and abiotic stress factors. Finding disease resistant wild barley genotypes facilitate disease resistance studies. In this current study, we determined *H. spontaneum* genotypes resistant to both forms of *P. teres*.

There are limited studies related to reactions of *H. spontaneum* genotypes to net blotch disease. In a study conducted by Kopahnke (1998), 770
Table 1- Seedling reactions of 104 Hordeum spontaneum genotypes to 3 virulent Pyrenophora teres f. sp. maculata isolates. Means not connected by same letter are significantly different (P<0.01).

<table>
<thead>
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<th>Genotype</th>
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<th>PTT**</th>
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**Note:** Means not connected by same letter are significantly different (P<0.01).
Table 1- (continued)- Seedling reactions of 104 Hordeum spontaneum genotypes to 3 virulent Pyrenophora teres f. maculata isolates and 3 virulent Pyrenophora teres f. teres isolates. Means not connected by same letter are significantly different (P<0.01)

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CV%: 9.41% 9.98% 9.58% 5.16% 4.46% 5.53%

*P. teres f. maculata scale values: 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, 9= S: susceptible. **P. teres f. teres scale values: 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, 10= VS: very susceptible
Hordeum spontaneum and 300 H. vulgare accessions were evaluated for their resistance status to P. teres under greenhouse and field conditions. H. spontaneum genotypes exhibited different resistance reactions and 143 genotypes showed resistant reaction to all isolates. Fetch et al (2003) determined the diversity of 116 H. spontaneum genotypes for their reaction to six barley fungal pathogens. The genotypes were obtained from Israel and Jordan. At seedling stage, a high level of diversity was found. Resistance frequency of genotypes from Israel and Jordan was high for net blotch (68% and 72%, respectively). Two genotypes were found resistant to 6 pathogens. Similarly, in our current study variation was found among the H. spontaneum genotypes. In our study, six H. spontaneum genotypes showed resistant to moderately resistant reactions to all Ptm and Ptt isolates. Jana & Bailey (1995) determined the resistance status of H. vulgare subsp. spontaneum and H. vulgare subsp. vulgare genotypes from Jordan and Turkey to P. teres f. maculata, P. teres f. teres and Cochliobolus sativus. More H. vulgare subsp. spontaneum genotypes were resistant to P. teres f. teres (21.8% vs. 0.5%) than H. vulgare subsp. vulgare. An equal number of H. vulgare subsp. spontaneum and H. vulgare subsp. vulgare genotypes were resistant to P. teres f. maculata. A larger percentage of H. vulgare subsp. spontaneum genotypes (10.5%) had at least moderate resistance to P. teres f. teres, P. teres f. maculata and C. sativus compared to only 1.3% in H. vulgare subsp. vulgare. However, in our current study, 25% of the genotypes and 7.6% of the genotypes exhibited resistant group reactions to P. teres f. maculata and P. teres f. teres, respectively. This finding is hopeful, because P. teres f. maculata is more prevalent in Turkey than P. teres f. teres (Karakaya et al 2014). H. spontaneum accessions showed different resistance reactions, depending upon their origin. Sato & Takeda (1997) evaluated net blotch resistance in 175 H. vulgare subsp. spontaneum (H. spontaneum) accessions and 149 wild Hordeum accessions of thirteen species or subspecies. Most H. spontaneum accessions showed resistance to each of the four P. teres f. teres isolates (Japanese isolates K105 and Pt860514 and Canadian isolates WRS102 and WRS1581) tested. Some accessions from Russia and Afghanistan showed a high level of resistance and Morocco accessions were susceptible. H. spontaneum accessions susceptible to the Canadian isolate WRS102 but resistant to the other three isolates were found in Iraq. This suggested the geographical differentiation of resistance genes in H. spontaneum. All accessions of the other wild Hordeum species, especially some accessions of H. marinum subsp. gussoneanum, showed high levels of resistance. Sato & Takeda (1997) concluded that resistance genes may be useful candidates for incorporation into cultivated barley.

H. spontaneum is a rich source of genes for disease resistance. Many resistant barley genotypes were found in barley evolution centers (Afanasenko et al 2000). Suitable habitat conditions for H. vulgare subsp. spontaneum exist especially in the Levant and Turkey and genetic diversity was observed in these populations (Jakob et al 2014). Turkey is an important gene center of barley and wild barleys (Kün 1996). Karakaya et al (2016) examined a total of 40 naturally growing H. spontaneum field populations in Şanlıurfa, Mardin, Şırnak, Siirt, Diyarbakır, Gaziantep, Kilis and Hatay provinces of Turkey for the presence of diseases and their severities in 2015. Nine H. spontaneum populations were disease free. The following diseases were found: Scald incited by Rhynchosporium commune, powdery mildew incited by Blumeria graminis f. sp. hordei, both forms of net blotch incited by Drechslera teres f. teres and D. teres f. maculata, semi loose smut incited by Ustilago nuda, brown rust (leaf rust) incited by Puccinia hordei and barley stripe caused by Drechslera graminea. Scald was the most commonly encountered disease followed by powdery mildew and net blotch. The incidence and severity values of diseases varied. The authors reported a wide range of variation in terms of disease resistance status of naturally growing H. spontaneum populations.
4. Conclusions

The use of disease resistant cultivars is the desirable control method of diseases. For sustainable crop production, monitoring virulence changes in pathogen is necessary. New pathotypes could be more virulent than previous pathotypes. For this reason, a broad base of genetical source is necessary. Wild barleys and especially *H. spontaneum* are valuable sources for disease resistance. Useful traits including disease resistance could be transferred to barley cultivars. Nevo (1992) pointed out the importance of *H. spontaneum* for disease resistant barley breeding programs and for developing a gene pool for desired traits. Also, Nevo et al (1986) examined the *H. spontaneum* populations of Israel, Turkey and Iran in the Fertile Crescent and reported their genetic diversity as well as their adaptability. Turkey is an important gene center of *Hordeum* species (Kün 1996). *Hordeum spontaneum* populations are naturally growing in Turkey and heterogenous nature of disease resistance among the populations has been reported (Karakaya et al 2016).

With this study, novel wild barley (*H. spontaneum*) genotypes resistant to both forms of *Pyrenophora teres* have been identified. These genotypes could be used in obtaining disease resistant and high yielding barley cultivars.

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