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# Identification of Barley Landraces and Wild Barley (*Hordeum* spontaneum) Genotypes Resistant to Rhynchosporium commune

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#### ABSTRACT

Barley scald is caused by the fungal pathogen *Rhynchosporium commune*. This disease causes substantial losses in barley production areas of the world. In this study, seedling resistance of 198 barley landraces, 104 wild barley (*Hordeum spontaneum*) genotypes and two susceptible Turkish cultivars (Bülbül 89 and Efes 3) to 6 *R. commune* isolates was assessed in greenhouse experiments. Virulence differences among the *R. commune* isolates were observed. One sixrowed barley landrace (Yeşilköy 9052) was resistant to all six isolates. Another six-rowed barley landrace (genotype no 17) showed resistant reaction to 5 isolates. Fourteen barley landraces were resistant and susceptible to 4 and 2 isolates, respectively. Twenty seven genotypes of *H. spontaneum* numbered as 5, 6, 7, 8, 9, 11, 16, 20, 30, 31, 36, 37, 48, 50, 51, 56, 58, 59, 60, 62, 67, 79, 80, 83, 90, 94 and 101 were found resistant to all six isolates of *R. commune*. Apart from these genotypes, 19 genotypes numbered as 1, 2, 32, 33, 34, 42, 43, 49, 52, 64, 66, 76, 77, 78, 96, 97, 102, 104 and 107 showed resistance to 5 isolates and susceptibility to only 1 isolate. Two susceptible Turkish cultivars Bülbül 89 and Efes 3 were found susceptible to 96% of the scald isolates. Resistant barley landraces and *Hordeum spontaneum* genotypes can be used as sources of resistance against *R. commune*.

Keywords: Scald; Rhynchosporium commune; Barley landraces; Wild barley; Hordeum spontaneum; Disease resistance

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#### **1. Introduction**

*Rhynchosporium commune* (formerly known as *Rhynchosporium secalis*) (Zaffarano et al 2011) is a haploid pathogen that attack different barley species including cultivated and wild barley. This pathogen can attack to barley plants and produce disease symptoms on leaves, leaf sheaths and ears of barley (Avora & Knogge 2012). The disease is common

in the world and disease losses on cultivated barley due to this pathogen have been reported from 10% to 70% (Shipton et al 1974; Eshetu 1985; Zhang et al 1992; Sheikh Jabbari 2008; Karakaya et al 2014). Control of barley scald disease is achieved by a variety of control measures including chemical, agronomical and biological control methods. Using resistant cultivars, due to environmentally friendly implementation of this control method, is desirable. However, to obtain scald disease resistance sources in barley, extensive studies of both fungus pathogenicity and barley cultivars/lines screening for resistance are required. Turkey is considered as one of the precious gene pools of barley including disease resistance sources in Fertile Crescent (Kün 1996; Badr et al 2000; Karakaya et al 2016). Barley landraces and wild barleys are important sources of genetic variation and disease resistance (Yitbarek et al 1998; Ceccarelli & Grando 2000; Ellis et al 2000). Barley landraces and wild barley (H. spontaneum) genotypes are widely distributed in Turkey (Kün 1996; Badr et al 2000; Karakaya et al 2016; Ergün et al 2017). Due to rich heritage of barley landraces and wild barley (H. spontaneum) genotypes in different sites of Turkey, finding new sources of scald disease resistance using barley landraces and wild barley genotypes and transferring resistance traits into cultivated barley could have a great value in scald disease resistance studies. In this study, seedling reactions of 198 barley landraces and 104 wild barley (H. spontaneum) genotypes were determined using 6 R. commune isolates.

# 2. Material and Methods

#### 2.1. Plant materials

Two hundred barley landraces and 107 *Hordeum spontaneum* genotypes were obtained from Central Research Institute for Field Crops located in Ankara, Turkey. These barley landraces and wild barley seeds were multiplied in the field for use in this study and in future studies. In the seed propagation process insufficient seeds were obtained from barley landraces no. 43 and no. 116 and wild barley genotypes numbered as 4, 15 and 41 and therefore, they were not used in the experiments.

#### 2.2. Rhynchosporium commune isolates

Six *Rhynchosporium commune* isolates [(4 most virulent isolates (GPS 71-U, 13GPS203, 13GPS207 and 13GPS 109), one isolate with high virulence value (13GPS149) and 1 isolate representing the most common pathotype (E4)] selected from Azamparsa (2015) study were used in determination of seedling

stage resistance of 198 barley landraces, 104 *H. spontaneum* genotypes and susceptible Turkish cultivars Bülbül 89 and Efes 3 under greenhouse conditions. Isolates GPS 71-U, 13GPS203, 13GPS207, 13GPS109, 13GPS149 and E4 were obtained from Gaziantep-Subağı, Manisa-Kula, İzmir-Bergama, Ankara-Şereflikoçhisar, Mardin-Midyat and Eskişehir-Tepebaşı regions of Turkey, respectively (Azamparsa 2015).

# 2.3. Making single spore isolates, inoculation, incubation and disease assessment

Infected leaf samples obtained from different regions of Turkey were cut into small sizes, surface sterilized with 70% ethyl alcohol in 15 seconds followed by 0.5% sodium hypochlorite in 90 seconds and placed on sterilized filter paper for 1 minute (Azamparsa 2015). These dried samples were placed on autoclaved Bean Agar (BA) medium (140 g fresh bean, 20 g dextrose, 18 g agar, 1 L distilled water) at 22 °C inside an incubator. Fungus colony was produced on this medium after 2-3 weeks. To obtain single spore of fungus, 1 mL of sterile water was placed in a small micro tube and by using a sterile needle small part of colony with spores was transferred into the micro tube. This micro tube was shaken well and with the use of a sterile loop spore suspension was dispersed on BA medium. After 2-3 days under a stereomicroscope germinated spores were taken to the other Petri dishes containing BA. Developed colonies of single spores were transformed to test tubes containing BA medium and stored at 4 °C in a refrigerator. To produce inoculum, each isolate was grown on BA medium for about 14 days and then distilled water was added onto this colony and spores were harvested. Harvested spore suspension was cleaned from large parts of colonies using a cheesecloth and spore concentration was adjusted to 1×10<sup>6</sup> spores mL<sup>-1</sup>. One drop of Tween-20 was added to each 100 mL of inoculum. Five to 10 seeds of each genotype were seeded to 7 cm diameter plastic pots. These pots were placed in the greenhouse and watered when needed. Inoculation of plants was made when plants produced 1.5 leaves. After inoculation, plants

were transferred inside of a moist chamber with 100% relative humidity and 16-17 °C temperature range for 48 hours to insure infection of plants. After 2 days, plants were taken into the greenhouse with 20-25 °C temperature range. Disease symptoms started 8-10 days after inoculation and first disease assessment was made using El-Ahmed (1981) scale after 14 days of inoculation. The second disease assessment was made four days later (18 days after inoculation) and results of second assessment were used in disease evaluation. Three replications were used. Scale values of 0-2.0 were considered as resistant reaction and scale values of 2.1-4.0 were considered as susceptible reaction.

# 3. Results and Discussion

Among barley landraces only one genotype (genotype no: 200, Yeşilköy 9052) was resistant to all 6 tested isolates and therefore it was determined as the most resistant genotype. Barley landrace no: 17 was resistant to 5 isolates and moderately susceptible to GPS71-U isolate and this genotype was rated as the second most resistant genotype. Fourteen barley landraces were resistant and susceptible to 4 and 2 isolates, respectively. These barley landraces included the following landraces: 2, 15, 20, 23, 24, 79, 81, 82, 83, 120, 121, 132, 134 and 194.

Presence of resistance to barley scald in barley landraces and cultivars were reported previously. In Ethiopia, up to 100 single plant derived lines of 18 barley landraces were tested against R. commune and it was concluded that on average landraces with late maturity showed more resistance to barley scald (Alemayehu & Parlevliet 1997). In another study in Ethiopia, 180 barley landraces and 600 single head plants selected from 60 promising populations were evaluated for barley scald disease resistance. Barley landraces from higher altitude were more resistant to R. commune (Yitbarek et al 1998). In Spain, Silvar et al (2010) used 159 (148 six-rowed and 11 two-rowed) inbred lines from local landraces and 16 commercial cultivars (8 six-rowed and 8 two-rowed) and tested their resistance against several barley diseases including barley scald. They

found resistant reaction in about 26% of Spanish lines (mostly winter barleys) to R. commune. In Turkey, some barley genotypes which were bred in breeding programs by Central Research Institute for Field Crops and International Center for Agriculture Research in Dry Areas (ICARDA) were tested against R. commune. One thousand and four hundred one and 1379 barley lines and cultivars were evaluated aginst barley scald in the field and greenhoue tests, respectively. From these tested lines and cultivars 432 genotypes in the field and 257 genotypes in the greenhouse were found resistant or moderately resistant to barley scald. From 15 barley cultivars only Erginel 90 cultivar was found resistant to R. commune in both field and greenhouse experiments (Albustan et al 1998). In the other experiment by Mert & Karakaya (2004) both differences for resistance among barley cultivars and pathogenicity among 5 isolates of R. commune were found. Among the tested cultivars Cetin 2000, Sahin 91, Erginel 90, Kıral 97, Akhisar 98, Çumra 2001 and Avci 2002 were found resistant to all isolates. Cultivars Bülbül 89 and Efes 3 were susceptible to barley scald isolates. In another study, reactions of 36 barley cultivars and 683 barley genotypes to R. commune were determined in greenhouse and field experiments and from 683 barley genotypes, 44% in greenhouse and 39% in field tests were found resistant to R. commune (Düşünceli et al 2008). There was significant correlation (r=0.53) between greenhouse and field test results (P=0.0001). The cultivars Avcı 2002, Çetin 2000, Kıral 97, Erginel 90, Akhisar 98, Kaya 7794, Yeşilköy 387 and Zafer 160 were found as resistant under both greenhouse and field conditions. The cultivars Vamikhoca, Çıldır 02 and Quantum were found as susceptible under greenhouse conditions; however, they were found as resistant under field conditions. Twenty five cultivars were susceptible to barley scald both in greenhouse and field tests. Azamparsa et al (2015a) reported virulence differences among the R. commune isolates. Isolate obtained from Gaziantep was the most virulent followed by isolates obtained from Manisa and Eskişehir provinces of Turkey. These results show differences in resistance among barley cultivars and genotypes as well as virulence

differences among isolates of *R. commune*. Similar results were obtained in our current study.

From 198 barley landraces which were tested in this study, 169 and 29 barley landraces were belonged to 2-rowed and 6-rowed barleys, respectively. While 6-rowed barley cultivars had medium disease value of 2.5 out of 4, two-rowed barley landraces had disease value of 3.3 out of 4. Based on these results it can be said that six-rowed barley landraces were more resistant than two-rowed barleys to *R. commune*. The same results were reached by many workers in previous studies. In these experiments six-rowed barley cultivars were found more resistant than two-rowed barley cultivars to *R. commune* (Zencirci & Hayes 1990; Mert & Karakaya 2004; Düşünceli et al 2008; Silvar et al 2010; Mert et al 2014).

Twenty seven genotypes of wild barley (*H. spontaneum*) numbered as 5, 6, 7, 8, 9, 11, 16, 20, 30, 31, 36, 37, 48, 50, 51, 56, 58, 59, 60, 62, 67, 79, 80, 83, 90, 94 and 101 were found resistant to all six isolates of *R. commune*. Apart from these genotypes, 19 genotypes numbered as 1, 2, 32, 33, 34, 42, 43, 49, 52, 64, 66, 76, 77, 78, 96, 97, 102, 104 and 107 showed resistance to 5 *R. commune* isolates and susceptibility to only 1 isolate.

Genotypes of H. spontaneum are used in breeding programs as resistance sources especially resistance to cold and dry weather and disease resistance (Chen et al 2008; Çelik & Karakaya 2017). In the current study 26% of wild barley genotypes showed resistance to barley scald disease in seedling stage. This result shows high potential of wild barley (H. spontaneum) genotypes for resistance to R. commune. Resistance genes were successfully transformed from wild barley to cultivated barley (Çelik & Karakaya 2017). Abbott et al (1992) screened accessions of H. vulgare ssp. spontaneum collected from Israel (70), Iran (15) and Turkey (6) against four isolates of R. commune in seedling stage and they found a high percentage of resistance (77%)among these wild barley accessions to barley scald. Cherif et al (2012) in Tunisia tested 56 accessions of wild barleys (H. spontaneum, H. marinum and H.

*murinum*) and 7 varieties of *H. vulgare* against net blotch and scald to find new sources of resistance to these diseases and they found sources of resistance to *R. commune* in three wild species of barley. However, due to strong incompatibility between cultivated and two wild barleys (*H. marinum* and *H. murinum*) (Pickering & Johnston 2005), it seems that transfer of scald resistance genes to cultivated barley from *H. spontaneum* is more feasible. In their study, two *H. spontaneum* accessions collected from Afghanistan and Egypt showed resistance to barley scald both in seedling and adult growth stages.

Two susceptible Turkish cultivars Bülbül 89 and Efes 3 used in the current study were found susceptible to 96% of the scald isolates. Susceptibility of these cultivars were reported previously (Mert & Karakaya 2004; Azamparsa et al 2015b) and confirmed with this study. Resistant *H. spontaneum* genotypes and barley landraces identified in this study could be used in breeding programs for obtaining scald resistant genotypes or promising landraces could be used directly as the seeding materials.

## 4. Conclusions

In this study only one barley landrace out of 198 landraces (genotype no: 200) (0.5% of the genotypes) showed resistance to all six tested isolates to R. commune. Another six-rowed barley (genotype no: 17) landrace showed resistant reaction to 5 isolates and moderately susceptible reaction to 1 isolate. Fourteen barley landraces were resistant and susceptible to 4 and 2 isolates, respectively. Twenty seven lines of wild barley (H. spontaneum) out of 104 genotypes were resistant to all 6 isolates of R. commune. In addition, 19 genotypes showed resistance to 5 isolates and susceptibility to only 1 isolate. Resistance to R. commune among barley landraces was low. On the other hand, more wild barley (H. spontaneum) genotypes were resistant to R. commune. It appears that Hordeum spontaneum genotypes are good resistance sources of scald disease of barley caused by *R. commune*.

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