Bitki Koruma Bülteni / Plant Protection Bulletin

http://dergipark.gov.tr/bitkorb

Original article

Determination of the pathotypes of *Rhynchosporium commune* (Zaffarona, McDonald & Linde) in some regions of Turkey

Türkiye'nin bazı bölgelerinde *Rhynchosporium commune* (Zaffarona, McDonald & Linde)'nin patotiplerinin belirlenmesi

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ARTICLE INFO

Article history:

DOI: 10.16955/bitkorb.460235

Received : 15.09.2018

Accepted : 30.05.2020

Keywords:

Rhynchosporium commune, differential set, scald pathotypes, *Hordeum vulgare*

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ABSTRACT

Barley scald, caused by the fungal pathogen *Rhynchosporium commune* is the most important disease of barley in the world and in Turkey. Surveys were conducted during 2012, 2013, and 2014 in different regions of Turkey. Isolations were accomplished from diseased leaves and from these fifty-two *R. commune* single spore isolates were selected. A total of 30 scald pathotypes were distinguished based on virulence on 17 barley differential set cultivars. Eighteen, 14, 6, and 1 of these pathotypes were from Central Anatolia, Southeast Anatolia, Aegean, and the Black Sea regions of Turkey, respectively. Twenty, 5, 1, 3, and 1 of these pathotypes were represented by 20, 2, 3, 4, and 7 isolates, respectively. None of the pathotypes was virulent on all 17 barley differential cultivars and two susceptible control cultivars. The most virulent pathotypes (pathotypes 29 and 30) were obtained from Manisa- Kula (13-203) and Gaziantep-Subağı (GPS71U) locations and the least virulent pathotype (pathotype 1) was obtained from Sivas-Gemerek (GPS31) and Sivas-Ulaş (NKT20) locations of Turkey. Among 17 barley differential cultivars, Jet and Abyssinia were susceptible to 1 pathotype, Osiris, Atlas 46, and Forrajera were susceptible to 3 pathotypes, La Mesita and Bey were susceptible to 7 pathotypes, Trebi was susceptible to 9 pathotypes, Pirate was susceptible to 10 pathotypes, Modoc was susceptible to 11 pathotypes, Kitchin and Igri were susceptible to 12 pathotypes, Armelle and Astrix were susceptible to 19 pathotypes, Athene was susceptible to 21 pathotypes, Steudelli was susceptible to 24 pathotypes and Digger was susceptible to 25 pathotypes. Among barley differential cultivars, Jet and Abyssinia cultivars were found as the most resistant, and Digger and Steudelli cultivars were the most susceptible cultivars. Two susceptible control cultivars Bülbül 89 and Efes 3 were found susceptible to 93% of scald pathotypes. It appears that considerable variation exists among the Turkish *R. commune* isolates obtained from some barley growing areas of Turkey.

INTRODUCTION

Barley (*Hordeum vulgare* L.) is one of the important cereal crops grown in the vast area of the world and Turkey. This crop is the second most important cereal in Turkey which is grown in 2.611.940 hectares of land, and 7.000.000 tonnes of yield was produced (TUİK 2018). Barley scald caused by *Rhynchosporium commune* Zaffarano, McDonald, and Linde (formerly *Rhynchosporium secalis* (Oudem.) J.J. Davis) (Zaffarano et al. 2011) is one of the important barley diseases in Turkey (Karakaya et al. 2014). Yield losses of 10%-70% due to this pathogen have been reported (Aktaş 1984, Sheikh Jabbari 2008, Shipton et al. 1974, Zhang et al. 1992). Barley scald is controlled using chemical, agronomical, and genetic resistance measures. Introducing new sources of resistance to scald is accomplished by screening barley genotypes as well as determining the degree of pathogenic variation in *R. commune* populations. This method may omit the control of this fungus by chemical measures and help to implement environmentally friendly ways of disease control. Knowing pathogenic variability and obtaining barley genotypes resistant to scald can lead to the prevention of disease losses. In this study, surveys were conducted during 2012, 2013, and 2014 in different regions of Turkey, and pathotypes of *R. commune* in some barley growing areas of Turkey were determined.

MATERIALS AND METHODS

Differential set for barley scald disease, previously used by Abang et al. (2006), was selected in this experiment. This differential set contained 17 barley scald differential cultivars were obtained from the International Center for Agricultural Research in the Dry Areas (ICARDA), The United States Department of Agriculture (USDA), and Dr. T. Fukuyama (Niigata University, Japan). Seeds were multiplied both in the field and under the greenhouse conditions. Additionally, two susceptible Turkish control cultivars (Bülbül 89 and Efes 3) (Azamparsa et al. 2015a, 2015b) were also used in our current study; but they were not used in pathotype identification.

During 2012, 2013, and 2014, *R. commune* surveys were conducted in some barley growing areas of Turkey. Samples were collected approximately at every 30 kilometers. Fields were inspected diagonally, or a zigzag pattern was followed. The size of the field was considered for sampling. At each sampling point, at least 10 plants were inspected (Aktaş 2001). Scald isolates were obtained from diseased leaves. These infected leaf samples showing characteristic scald symptoms were cut into small sizes, surface-sterilized 15 seconds with 70% ethyl alcohol followed by 90 seconds 5% sodium hypochlorite, and finally placed on sterilized filter paper 1 minute for drying. These infected dried samples were placed on Bean Agar (BA) medium (140 g fresh bean, 20 g dextrose, 18 g agar, 1 l distilled water) or Potato Dextrose Agar medium at 22±1 °C inside an incubator. Fungus colony was produced on these media after 2-3 weeks. To produce single spores of the fungus, 1 ml of sterile water was placed in small microtubes, and by using a sterile needle, a small part of the colony with spores was transferred into microtubes and then the microtubes were shaken well. With the use of sterile loops, spore suspensions were placed on BA medium and these spores were spread on BA medium. After 2-3 days, under a stereomicroscope, germinated spores were taken to the Petri dishes containing BA. Developed colonies of single spores were transformed to test tubes containing BA medium and stored at 4 °C in the refrigerator. From these isolates, 52 single spore isolates representing different regions of Turkey were selected.

To produce inoculum, each isolate was grown on BA medium for about 14 days, then sterile distilled water was added onto the colony. Spores were collected using a cover slide. In harvested single spore suspensions, large parts of colonies were removed using a sterile cheesecloth. Finally, the spore concentration of 1×10^6 spores/ml was adjusted using a hemocytometer (Abang et al. 2006, Mert and Karakaya 2003). One drop of Tween-20® was added to every 100 ml of inoculum.

Differential set cultivars and two susceptible control cultivars were planted into plastic pots (7x7x9 cm) containing soil: sand: organic matter (60: 20: 20). Five to seven seeds were placed into each pot. Three replications were arranged in a randomized block design. Inoculation of plants was made when plants produced 1.5 leaves (Zadoks scale 11-12) (Zadoks et al. 1974). After inoculation, plants were transferred to a moist chamber with 100% relative humidity and 16-17 °C for 48 hours to ensure infection of plants. After 2 days, plants were taken to the greenhouse with a 22-25 °C temperature range. Plants were watered as necessary. Disease symptoms were visible in 8-10 days after inoculation. The first disease assessment was made using El-Ahmed (1981) 0-4 scale after 14 days of inoculation. The second disease assessment was made four days later (18 days after inoculation), and the results of the second assessment were used in disease evaluation. Scale values 0-2 were considered as resistant, and scale values 2.01-4 were considered as susceptible.

RESULTS

Seventeen barley scald differential cultivars and two susceptible barley control cultivars were inoculated using 52 single spore isolates of *R. commune*. Resistance or susceptibility reactions of these cultivars were distinguished using a 0-4 scale (El-Ahmed 1981) (Table 1).

Table 1. Reactions of 17 barley scald differential set cultivars and two susceptible Turkish cultivars to 52 isolates of *Rhynchosporium commune*. For evaluation, a 1-4 scale was used (Al-Ahmed (1981). Scale values 0-2 were considered as resistant and scale values 2.01-4 were considered as susceptible

			Barley scald differential set genotypes[1]																		Bülbül 89	Efes 3
No	Isolate No.	Location	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	Mean	18	19
1	GPS31	Sivas-Gemerek	0.3	0.3	0.0	0.0	0.3	0.0	0.0	0.3	0.7	0.0	0.3	0.7	0.3	0.0	0.0	0.0	0.0	0.2	1.3	1.7
2	NKT20	Sivas-Ulaş	0.0	0.0	0.0	1.7	0.0	0.0	0.0	1.0	0.0	0.0	0.0	1.7	0.0	0.0	0.7	0.7	0.0	0.3	3.0	3.7
3	13-160	Diyarbakır-Central	1.0	1.3	0.0	0.0	2.0	0.0	0.0	2.0	2.0	0.0	1.3	2.7	1.7	0.3	2.0	0.0	1.3	1.0	3.0	3.7
4	13-144	Mardin-Midyat	0.0	0.0	3.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.0	0.0	0.0	0.0	0.0	0.0	0.4	3.3	3.7
5	GPS87	Çankırı-Central	1.3	1.3	3.7	0.0	0.0	0.0	0.0	1.3	0.0	0.0	0.0	3.0	0.0	0.0	0.0	0.0	0.0	0.6	3.7	4.0
6	13-147	Mardin- Midyat	1.0	0.3	2.7	2.0	0.0	0.0	0.0	3.0	0.0	0.0	0.0	1.0	0.0	0.0	0.0	0.0	0.0	0.6	1.7	2.0
7	GPS93	Ankara-Polatlı	1.3	2.0	0.0	0.3	0.0	0.0	0.0	2.7	0.0	0.0	0.0	2.3	1.0	0.0	1.0	0.0	0.0	0.6	2.7	3.3
8	GPS110	Konya-Meram	1.7	1.3	0.7	0.0	0.7	0.3	0.0	2.3	0.0	0.0	0.0	3.0	0.7	0.0	0.3	0.0	1.0	0.7	3.0	3.0
9	13-122	Şanlıurfa-Central	1.3	1.7	2.0	2.0	0.0	0.0	0.0	2.7	0.0	0.0	0.0	2.7	0.0	0.0	1.7	1.7	0.0	0.9	3.0	3.7
10	13-202	Uşak-Central	0.0	0.0	2.0	1.3	1.7	1.7	0.0	3.0	1.0	0.0	1.3	3.0	2.0	0.0	1.0	1.7	0.0	1.2	3.0	3.0
11	GPS66	Kırşehir-Central	3.3	4.0	3.3	2.0	0.0	0.0	1.3	2.0	0.3	0.0	0.0	1.7	0.0	0.0	0.3	0.0	0.0	1.1	4.0	4.0
12	GPS65	Nevşehir-Hacıbektaş	3.7	3.0	0.7	0.3	1.0	0.0	0.0	3.0	0.0	0.0	0.3	1.7	0.0	0.0	0.0	0.0	0.0	0.8	2.7	3.7
13	13-150	Mardin-Midyat	1.0	1.3	4.0	0.7	0.0	0.0	1.0	3.7	0.0	0.0	0.0	3.3	0.0	0.0	0.0	0.0	0.0	0.9	4.0	4.0
14	13-126	Şanlıurfa-Viranşehir	1.0	1.0	3.7	2.0	0.0	0.0	0.3	4.0	0.0	0.0	0.0	3.7	0.0	0.0	0.3	0.0	0.0	0.9	3.7	4.0
15	13-157S	Diyarbakır-Central	1.3	0.7	0.0	1.0	1.7	0.0	0.0	2.0	3.0	1.7	1.3	3.0	3.0	0.0	1.7	0.0	0.7	1.2	3.0	3.0
16	13-117	Niğde-Ulukışla	3.7	3.3	2.3	1.3	0.0	0.3	0.7	3.3	1.0	0.0	0.0	0.3	0.3	0.0	0.7	0.0	0.0	1.0	3.7	4.0
17	13-197	Aksaray-Central	2.7	3.0	1.7	1.3	0.0	1.3	0.3	2.7	0.3	1.0	3.0	1.3	1.7	1.0	1.3	1.0	0.7	1.4	3.0	3.7
18	Department	Ankara- Dışkapı	3.3	3.0	1.0	1.7	0.0	0.0	0.0	3.7	0.3	0.0	0.0	3.0	0.0	0.3	1.0	0.3	0.0	1.0	3.0	3.7
19	GPS127	Konya-Selçuklu	3.7	3.7	2.0	1.3	0.0	0.0	0.0	3.7	0.0	0.0	0.0	3.0	0.0	0.0	0.3	0.0	0.0	1.0	3.3	4.0
20	13-154	Mardin-Midyat	3.0	3.0	2.0	1.7	1.0	0.3	0.3	3.7	0.3	0.0	0.0	3.7	0.3	0.3	1.0	0.3	0.0	1.2	2.3	4.0
21	13-209	İzmir-Menderes	4.0	3.7	2.0	1.3	0.0	0.0	0.7	4.0	0.3	0.0	0.7	3.7	0.0	0.7	0.3	0.0	0.0	1.3	2.3	4.0
22	NKT29	Sivas-Şarkışla	3.3	3.0	3.3	1.0	0.0	0.0	1.3	2.3	0.0	0.0	1.0	2.3	0.3	0.0	0.0	0.0	0.0	1.1	3.7	3.7
23	GPS71	Kırşehir-Kaman	3.0	3.0	2.7	1.0	0.7	0.3	0.7	3.7	0.7	0.0	0.0	3.0	0.3	0.3	0.7	0.0	0.0	1.2	3.0	4.0
24	GPS76	Ankara-Kalecik	3.7	3.3	4.0	1.0	0.0	0.3	1.3	3.7	0.7	0.0	1.7	3.0	0.3	1.0	0.3	0.0	0.0	1.4	4.0	4.0
25	13-130	Şanlıurfa-Ceylanpınar	0.0	0.0	4.0	4.0	0.3	2.0	4.0	4.0	0.0	0.0	1.0	4.0	0.0	0.0	0.0	0.0	0.0	1.4	4.0	4.0
26	GPS100	Konya- Tuzlukçu	4.0	4.0	3.7	3.0	0.7	0.0	1.7	2.3	0.7	0.0	0.7	4.0	0.0	0.3	1.0	0.0	0.0	1.5	4.0	4.0
27	GPS4U	Ankara Çankaya	4.0	4.0	3.3	3.0	0.3	0.7	1.7	4.0	0.7	0.0	0.3	3.0	0.0	0.3	1.0	0.0	0.0	1.5	4.0	4.0
28	13-188	Kayseri- İncesu	4.0	4.0	4.0	3.3	0.0	0.0	1.0	3.7	0.0	0.0	2.0	4.0	0.0	0.3	0.7	0.0	0.0	1.6	3.7	4.0
29	GPS106	Konya-Beyşehir	4.0	4.0	4.0	3.0	0.7	0.0	1.7	3.7	1.0	0.0	1.3	3.7	0.0	0.3	1.3	0.0	0.0	1.7	4.0	4.0
30	E4	Eskişehir-Tepebaşı	4.0	3.3	3.7	3.0	1.0	0.3	1.3	4.0	1.7	0.0	1.3	4.0	0.0	1.3	0.3	0.0	0.0	1.7	3.0	4.0
31	13-194	Kayseri-İncesu	3.7	3.0	3.3	3.3	1.3	0.0	1.3	4.0	1.3	0.0	1.3	4.0	0.3	1.0	2.0	0.0	0.0	1.8	3.0	4.0
32	GPS120	Konya-Güneysınır	4.0	3.3	3.7	3.3	1.0	0.3	1.3	4.0	0.7	0.0	1.7	4.0	0.0	1.3	1.3	0.0	0.3	1.8	3.7	4.0
33	Hay1Ank	Ankara-Haymana	3.7	3.7	3.0	1.7	0.0	0.0	0.7	4.0	0.0	0.0	0.3	3.0	0.0	0.0	3.0	0.0	0.0	1.4	3.7	4.0
34	E85	Eskişehir-Sivrihisar	0,0	0.0	0.7	0.0	3.3	3.0	0.0	2.3	1.7	1.3	1.7	3.0	3.3	0.0	3.7	1.0	1.0	1.5	3.0	3.3
35	13-208	Manisa-Akhisar	4.0	4.0	3.7	3.7	0.0	0.0	2.3	3.0	1.3	0.0	1.3	4.0	0.0	0.0	1.0	0.0	0.0	1.7	4.0	4.0
36	13-152	Mardin- Midyat	4.0	4.0	4.0	4.0	0.0	0.0	3.7	4.0	1.0	0.0	1.0	4.0	0.0	1.0	1.0	0.0	0.0	1.9	4.0	4.0
37	GPS60	Yozgat- Yenifakılı	4.0	4.0	4.0	4.0	0.7	1.3	3.7	4.0	1.3	0.0	1.3	4.0	0.0	0.0	2.0	0.0	0.0	2.0	4.0	4.0
38	E43	Eskişehir –Sarıcakaya	4.0	4.0	4.0	4.0	1.0	0.0	3.7	4.0	0.3	0.0	1.7	4.0	0.0	2.0	1.7	0.0	0.0	2.0	4.0	4.0
39	E97	Eskişehir-Sivrihisar	4.0	4.0	4.0	3.0	0.0	0.0	3.0	3.3	1.3	0.3	2.3	4.0	1.0	1.7	1.3	0.0	0.0	2.0	4.0	4.0
40	14-120	Kastamonu- Devrekani	4.0	4.0	4.0	3.7	0.0	0.0	2.7	4.0	1.7	0.0	2.3	4.0	0.0	1.0	2.0	0.0	0.0	2.0	4.0	4.0
41	E1	Eskişehir-Tepebaşı	4.0	4.0	3.7	3.7	1.0	0.3	1.7	4.0	2.7	0.0	3.0	4.0	0.0	1.7	1.7	0.0	0.0	2.1	3.3	4.0
42	GPS73	Ankara-Akyurt	4.0	4.0	4.0	4.0	0.0	0.0	3.0	4.0	1.3	0.3	3.0	4.0	0.7	3.3	1.3	0.0	0.0	2.2	4.0	4.0
43	13-149	Mardin-Midyat	4.0	4.0	4.0	4.0	0.3	0.3	3.3	4.0	1.7	0.0	3.3	4.0	1.0	2.3	1.3	0.0	0.3	2.2	4.0	4.0
44	GPS2	Ankara-Lalahan	4.0	4.0	4.0	4.0	1.3	0.0	3.0	4.0	2.3	0.0	1.7	4.0	0.3	0.0	3.0	0.0	0.0	2.1	4.0	4.0
45	13-157	Diyarbakır-Central	3.0	3.7	2.0	1.7	3.7	1.7	1.0	3.0	4.0	2.0	4.0	4.0	4.0	0.0	4.0	1.7	1.3	2.6	3.7	3.7
46	13-153	Mardin-Midyat	4.0	4.0	4.0	4.0	1.3	0.0	4.0	4.0	1.7	0.0	4.0	4.0	1.0	3.7	3.7	0.0	1.0	2.6	4.0	4.0
47	13-177	Adıyaman-Gölbaşı	4.0	4.0	4.0	4.0	0.3	0.0	3.3	3.7	2.7	0.0	2.3	4.0	0.3	0.0	3.0	0.0	0.0	2.1	4.0	4.0
48	13-204	Manisa-Kula	3.7	3.0	3.7	1.3	3.0	0.0	0.3	3.0	3.7	2.0	3.7	3.0	3.7	1.7	3.0	0.7	0.3	2.3	3.7	4.0
49	13-207	İzmir- Bergama	4.0	4.0	4.0	4.0	2.7	0.0	4.0	4.0	2.0	0.0	3.7	4.0	2.0	4.0	2.7	0.0	0.0	2.6	4.0	4.0
50	13-109	Ankara-Şereflikoçhisar	3.0	3.7	3.0	1.3	4.0	1.3	0.0	4.0	3.7	0.0	3.7	4.0	4.0	0.0	3.7	2.3	1.0	2.5	4.0	4.0
51	13-203	Manisa- Kula	2.0	2.0	4.0	4.0	4.0	4.0	3.0	4.0	4.0	3.0	4.0	4.0	4.0	0.0	4.0	4.0	2.0	3.3	4.0	4.0
52	GPS71U	Gaziantep-Subağı	0.0	0.0	4.0	4.0	4.0	3.7	4.0	4.0	3.0	1.3	4.0	3.7	3.0	0.0	3.0	3.7	3.7	2.9	4.0	4.0
		Mean	2.8	2.7	2.8	2.2	0.9	0.5	1.4	3.2	1.1	0.2	1.4	3.2	0.8	0.6	1.4	0.4	0.3		3.5	3.8

[1] Differential cultivars and susceptible control cultivars: 1=Armelle, 2= Astrix, 3= Athene, 4= Igri, 5= La Mesita, 6= Osiris, 7= Pirate, 8= Digger, 9= Trebi, 10= Jet, 11= Kitchin, 12= Steudelli, 13= Bey, 14= Atlas 46, 15= Modoc, 16= Forrajera, 17= Abyssinia, 18= Bülbül 89, 19= Efes 3

Table 2. Numbers of diseased genotypes, susceptible differential set cultivars and pathotype numbers of selected 52 isolates of *Rhynchosporium commune* on 17 barley differential cultivars

No.	Isolate No.	Location	No. of diseased genotypes	Susceptible cultivars	Pathotype No.
1	GPS31	Sivas-Gemerek	0	0	1
2	NKT20	Sivas-Ulaş	0	0	1
3	13-160	Diyarbakır-Central	1	12	2
4	13-144	Mardin-Midyat	2	3,12	3
5	GPS87	Çankırı-Central	2	3,12	3
6	13-147	Mardin- Midyat	2	3,8	4
7	GPS93	Ankara-Polatlı	2	8,12	5
8	GPS110	Konya-Meram	2	8,12	5
9	13-122	Şanlıurfa-Central	2	8,12	5
10	13-202	Uşak-Central	2	8,12	5
11	GPS66	Kırşehir-Central	3	1,2,3	6
12	GPS65	Nevşehir-Hacıbektaş	3	1,2,8	7
13	13-150	Mardin-Midyat	3	3,8,12	8
14	13-126	Şanlıurfa-Viranşehir	3	3,8,12	8
15	13-157H	Diyarbakır-Central	3	9,12,13	9
16	13-117	Niğde-Ulukışla	4	1,2,3,8	10
17	13-197	Aksaray-Central	4	1,2,8,11	11
18	Department	Ankara- Dışkapı	4	1,2,8,12	12
19	GPS127	Konya-Selçuklu	4	1,2,8,12	12
20	13-154	Mardin-Midyat	4	1,2,8,12	12
21	13-209	İzmir-Menderes	4	1,2,8,12	12
22	NKT29	Sivas-Şarkışla	5	1,2,3,8,12	13
23	GPS71	Kırşehir-Kaman	5	1,2,3,8,12	13
24	GPS76	Ankara-Kalecik	5	1,2,3,8,12	13
25	13-130	Şanlıurfa-Ceylanpınar	5	3,4,7,8,12	14
26	GPS100	Konya- Tuzlukçu	6	1,2,3,4,8,12	15
27	GPS54U	Ankara-Çankaya	6	1,2,3,4,8,12	15
28	13-188	Kayseri-İncesu	6	1,2,3,4,8,12	15
29	GPS106	Konya-Beysehir	6	1,2,3,4,8,12	15
30	E4	Eskişehir-Tepebaşı	6	1,2,3,4,8,12	15
31	13-194	Kayseri-İncesu	6	1,2,3,4,8,12	15
32	GPS120	Konya-Güneysınır	6	1,2,3,4,8,12	15
33	Hay1-Ankara	Ankara-Haymana	6	1,2,3,8,12,15	16
34	E85	Eskişehir-Sivrihisar	6	5,6,8,12,13,15	17
35	13-208	Manisa-Akhisar	7	1,2,3,4,7,8,12	18
36	13-152	Mardin- Midyat	7	1,2,3,4,7,8,12	18
37	GPS60	Yozgat- Yenifakılı	7	1,2,3,4,7,8,12	18
38	E43	Eskişehir -Sarıcakaya	7	1,2,3,4,7,8,12	18
39	E97	Eskişehir-Sivrihisar	8	1,2,3,4,7,8,11,12	19
40	14-120	Kastamonu- Devrekani	8	1,2,3,4,7,8,11,12	19
41	E1	Eskişehir-Tepebaşı	8	1,2,3,4,8,9,11,12	20
42	GPS73	Ankara-Akyurt	9	1,2,3,4,7,8,11,12,14	21
43	13-149	Mardin-Midyat	9	1,2,3,4,7,8,11,12,14	21
44	GPS2	Ankara-Lalahan	9	1,2,3,4,7,8,9,12,15	22
45	13-157	Diyarbakır-Central	9	1,2,5,8,9,11,12,13,15	23
46	13-153	Mardin-Midyat	10	1,2,3,4,7,8,11,12,14,15	24
47	13-177	Adıyaman-Gölbaşı	10	1,2,3,4,7,8,9,11,12,15	25
48	13-204	Manisa-Kula	10	1,2,3,5,8,9,11,12,13,15	26
49	13-207	İzmir- Bergama	11	1,2,3,4,5,7,8,11,12,14,15	27
50	13-109	Ankara-Şereflikoçhisar	11	1,2,3,5,8,9,11,12,13,15,16	28
51	13-203	Manisa- Kula	13	3,4,5,6,7,8,9,10,11,12,13,15,16	29
52	GPS71U	Gaziantep-Subağı	13	3,4,5,6,7,8,9,11,12,13,15,16,17	30

[1]Differential cultivars: 1-Armelle, 2- Astrix, 3- Athene, 4- Igri, 5- La Mesita, 6- Osiris, 7- Pirate, 8- Digger, 9-Trebi, 10- Jet, 11- Kitchin, 12- Steudelli, 13- Bey, 14- Atlas 46, 15- Modoc, 16- Forrajera, 17- Abyssinia

The virulence of 52 *R. commune* isolates on 17 barley differential cultivars were evaluated, and 30 different pathotypes of *R. commune* were determined (Table 2). Among these pathotypes, pathotype no: 29 (Manisa-Kula, 13-203) (Figure 1) and pathotype no:30 (Gaziantep-Subağı, GPS71-U) were determined as the most virulent isolates of *R. commune* based on disease symptoms on 17 scald differential cultivars. On the other hand, isolates GPS31 (Sivas-Gemerek) and NKT20 (Sivas-Ulaş) were grouped as pathotype 1, and showed the least virulent reactions on differential set cultivars. These two isolates did not produce any disease symptoms on most of the differential set cultivars; however, they produced disease symptoms on two susceptible control cultivars Bülbül 89 and Efes 3. Based on disease reactions on two susceptible control cultivars, different reactions were detected. Isolate GPS31 produced lower disease (scale values 1.3 and 1.7 for Bülbül 89 and Efes 3, respectively) than the other isolate (NKT20). Both control cultivars showed susceptible reactions to isolate NKT20. These two susceptible control cultivars were not included in the barley scald differential set and they were not included in the pathotype determination of isolates of *R. commune*. Isolate 13-147 caused susceptible reactions on barley differential cultivars Athene (scale value 2.7) and Digger (scale value 3.0), however, this isolate produced less disease symptoms on two susceptible control cultivars Bülbül 89 (scale value 1.7) and Efes 3 (scale value 2.0). Each of twenty pathotypes was represented by one isolate and pathotype no: 15 was represented by 7 isolates. Pathotype no: 15 caused virulent reactions on Armelle, Astrix, Athene, Igri, Digger, and Steudelli cultivars and it was determined as the most widespread pathotype among the all pathotypes. All of these 7 isolates were obtained from Central Anatolia region of Turkey. Other pathotypes were represented by 2, 3, and 4 isolates (Tables 1 and 2).

Figure 1. Symptoms caused by *Rhynchosporium commune* isolate 13-203 (pathotype 29) on Digger (left), Trebi (center), and Osiris (right) barley scald differential set cultivars

In this study, 30 isolates were taken from Central Anatolia region including Ankara (8), Çankırı (1), Kayseri (2), Eskişehir (5), Kırşehir (2), Konya (5), Nevşehir (1), Niğde (1), Sivas (3), Yozgat (1), and Aksaray (1) locations. From Southeast Anatolia region 15 isolates were selected from Adıyaman (1), Diyarbakır (3), Gaziantep (1), Mardin (7) and Şanlıurfa (3) locations. Six isolates were taken from Aegean region that included İzmir (2), Manisa (3) and Uşak (1) locations and from Black Sea region one isolate from Kastamonu location was selected. Among the differential set cultivars, Jet and Abyssinia cultivars showed the most resistant reactions against 52 isolates of *R. commune*. Jet and Abyssinia cultivars showed susceptible reactions to pathotypes 29 and 30, respectively. Both pathotypes, 29 and 30, showed the most virulent reactions on barley differential set cultivars and susceptible control cultivars. Only one isolate produced susceptible reactions on these two barley differentials and therefore, in this study these two cultivars were determined as the most resistant cultivars to *R. commune*. The two susceptible control cultivars Bülbül 89 and Efes 3 were found as susceptible to 50 isolates out of 52 isolates of *R. commune*. In addition to these two control cultivars, Digger and Steudelli differential set cultivars were determined as susceptible to 45 isolates (87%) out of 52 isolates. These results showed that Digger and Steudelli were the most susceptible differential set cultivars (Table 1).

DISCUSSION

Fifty-two isolates of *R. commune* were tested on 17 barley scald differential cultivars and two susceptible control cultivars (cultivar Bülbül 89 and cultivar Efes 3) and 30 pathotypes were determined (Tables 1 and 2).

With respect to regions in which isolates were taken, in Aegean region 6 different pathotypes were determined from 6 isolates of *R. commune* (Tables 1 and 2). In Southeast Anatolia region 14 pathotypes from 15 isolates were determined. Two isolates from Mardin-Midyat (GPS 13-150) and from Şanlıurfa-Viranşehir (GPS 13-126) belonged to the same pathotype (pathotype 3). Eighteen different pathotypes were identified from 30 Central Anatolia region isolates. Pathotype numbers 1 [Sivas-Gemerek (GPS31) and Sivas-Ulaş (NKT20)], 5 [Ankara-Polatlı (GPS93) and Konya-Meram (GPS110)], 12 [Ankara-Dışkapı (Department) and Konya-Selçuklu (GPS127)] and 18 [Eskişehir-Sarıcakaya (E43) and Yozgat-Yenifakılı (GPS60)] were represented with isolates taken from 2 different locations. Pathotype no: 13 was identified from 3 different locations [Ankara-Kalecik (GPS76), Kırşehir-Kaman (GPS71) and Sivas-Şarkışla (NKT29)] and pathotype no: 15 was represented with isolates from 7 different locations [Ankara (GPS54U), Eskişehir-Tepebaşı (E4), Kayseri-İncesu (13-188), Kayseri-İncesu (13-194), Konya-Tuzlukçu (GPS100), Konya-Beyşehir (GPS106) and Konya-Güneysınır (GPS120)]. The other remaining 20 pathotypes were represented with one isolate. In our current research, among the barley scald differential set, Jet and Abyssinia were found as the most resistant cultivars. Only one isolate was virulent on these cultivars. On the other hand, cultivars Digger and Steudelli were susceptible to 45

isolates that were rated as the most susceptible cultivars after two control susceptible cultivars Bülbül 89 and Efes 3.

Although some studies showed similarities to our current study, some other studies presented different results. In Norway, Jet cultivar was found resistant to 8 isolates out of 11 isolates of *R. secalis* (Reitan et al. 2002). In Japan, among 58 pathotypes, Jet and Abyssinian cultivars were identified as resistant to 39 and 46 pathotypes of R. secalis, respectively. On the other hand, Steudelli cultivar was determined as susceptible to the majority of pathotypes (Takeuchi and Fukuyama 2009). In Tunisia, Bouajila et al. (2006) reported Abyssinia cultivar as resistant to 66.7% of the pathotypes. Additionally in Tunisia, Steudelli cultivar was reported as more resistant than Jet cultivar to *R. secalis* (Bouajila et al. 2010). In that study, among the 75 pathotypes tested, Steudelli and Jet cultivars were resistant to 47 and 35 pathotypes, respectively. Although both Steudelli and Jet cultivars have possessed the same recessive genes *rh6* and *rh7* in common as Bouajila et al. (2010) mentioned, however, they showed different reactions in different studies. This may show that there might be other resistance gene or genes involving resistance in these two cultivars. In the study conducted by Bouajila et al (2010), Kitchin and Abyssinian cultivars were resistant to 39 and 51 pathotypes of *R. secalis* out of 75 pathotypes, respectively. In our study, Kitchin cultivar exhibited resistance to 18 pathotypes (60%). Cultivar Kitchin showed better performance of resistance (87%) against scald pathotypes in Canada (Xi et al. 2002). In Japan, Kitchin cultivar showed resistance to 17 pathotypes out of 58 pathotypes (Takeuchi and Fukuyama 2009). In our study, Osiris and Forrajera cultivars were tested using 52 isolates of *R. commune* and they showed resistant reaction to 49 isolates. These cultivars showed susceptible reactions to only 3 isolates. Therefore, Osiris and Forrajera cultivars were determined as the most resistant cultivars to *R. commune* following the cultivars Jet and Abyssinia. These cultivars could be used in scald resistance breeding programs in Turkey for obtaining scald resistant barley genotypes. In Italy, Ceoloni (1980) tested the most virulent and the most prevalent race RC1 on 13 barley scald differential genotypes and this race was virulent on 10 of these genotypes. In this study, Atlas, Atlas 46, and Osiris cultivars were resistant to all isolates. In our study, cultivar Atlas 46 was tested using 52 *R. commune* isolates and this cultivar was found susceptible to only 4 isolates (7.6%) or 3 pathotypes (9.6%) and rated as one of the most resistant cultivars to *R. commune*. In Australia (Ali et al. 1976, Brown 1985), Italy (Ceoloni 1980), Canada (Tekauz 1991, Xi et al. 2002, Xue et al. 1991), and Norway (Reitan et al. 2002) Atlas 46 cultivar which possesses *Rrs1* and *Rrs2* resistance genes showed resistant reaction against all isolates of *R. secalis*. In another study, Atlas 46 cultivar showed a resistant reaction to 72 isolates out of 100 isolates and exhibited a susceptible reaction to the rest of 28 *R. secalis* isolates (Bouajila et al. 2006). In Denmark, 38 isolates of *R. secalis* were studied using 23 barley genotypes and the Osiris genotype was determined resistant to all isolates (Lyngs Jorgenson and Smedegaard-Petersen 1995). In another study conducted in Norway, Salamati and Tronsmo (1997) assessed the reactions of 42 *R. secalis* isolates on barley genotypes. In their study, the only Osiris cultivar was resistant to all isolates tested. Cultivars Modoc, Kitchin, and Abyssinian reacted intermediately (score type 3 reaction) to most of the matching isolates. Cultivar La Mesita was susceptible to all isolates. In our study, cultivar La Mesita showed susceptibility to 7 isolates (13.46%) and the Modoc cultivar was susceptible to 11 isolates (21.15%). In Denmark (Lyngs Jorgensen and Smedegaard-Petersen 1995) and England (Jones et al. 1993) cultivar Osiris was found as the most resistant cultivar and in the USA (Goodwin et al. 1992) the same cultivar was determined as the second most resistant cultivar. In another study conducted in Norway (Reitan et al. 2002), Osiris was the most resistant cultivar against 11 isolates of *R. secalis*. In Canada, reactions of 83 barley lines and scald differential set genotypes were tested against 4 pathotypes of *R. secalis* in the field and Osiris, Abyssinian, and Turkish cultivars were found as the most resistant cultivars (Turkington and Xi 2005). In Turkey, 50 single spore isolates of *R. secalis* from different regions were tested on 10 barley differentials and 41 pathotypes were determined (Araz and Maden 2006). The researchers determined that Osiris was susceptible to 1 pathotype and resistant to 40 pathotypes. In their study, cultivars Steudelli, Modoc, and Kitchin showed susceptible reactions to 12, 21, and 18 pathotypes out of 41 pathotypes, respectively. Another study in Hokuriku region of Japan by Fukuyama et al. (1998), among barley genotypes, Osiris and C.I. 3515 were found as the most resistant genotypes against 107 isolates of scald. For this reason, Osiris and C.I. 3515 genotypes were introduced as resistance sources to *R. secalis*. In Hokuriku and Tohoku regions of Japan (Takeuchi and Fukuyama 2009), probability of having more resistance genes than known Osiris resistance genes (*Rrs4*, *rrs6*, and *Rh10*) were emphasized, and use of this cultivar as a resistance source was recommended. In Canada, under field conditions, 41 barley cultivars and 9 barley scald differential set genotypes were tested against scald disease. Osiris cultivar was recognized as the most resistant cultivar and significant role of *Rh4* gene in resistance was determined (Sorkhilaleloo et al. 2010). In contrast to results mentioned above, using 100 isolates of *R. secalis* on 19 barley scald differential set genotypes determined that Osiris cultivar was susceptible to 73% of the isolates (Bouajila et al. 2006).

In addition, Rihane, and La Mesita cultivars with 69% and 61% susceptibility to scald were determined. In our study, Armelle and Astrix cultivars showed susceptible reactions to 35 isolates (67.3%), and Igri cultivar was susceptible to 23 (44.2%) isolates of *R. commune*. In contrast to our results, Bouajila et al. (2006) reported that the Astrix cultivar was the most resistant cultivar with a resistant reaction to 77 pathotypes (82.7%) out of 93 pathotypes. In their study, La Mesita and Digger cultivars were found as the most susceptible cultivars with susceptible reactions to 74% and 72% of pathotypes, respectively. In another research carried out in Tunisia, Astrix cultivar was determined as the most resistant cultivar against 75 pathotypes of *R. secalis* (Bouajila et al. 2010). In a study performed by Abang et al. (2006), 8 isolates tested using barley scald differential cultivars, Armelle, Astrix and Atlas 46 cultivars were found as the most resistant cultivars against all isolates, and Digger cultivar was the most susceptible cultivar to all isolates. In their study, Igri, La Mesita, Jet, and Forrajera cultivars showed susceptible reactions to 6 isolates (76% of isolates) of *R. secalis*. Additionally, in the same study, Osiris and Steudelli genotypes were found as susceptible against 4 isolates (50% of isolates). Abyssinia cultivar was found as susceptible to 2 isolates and resistant to the 6 isolates. In contrast to Abang et al. (2006) study, Arabi et al. (2008) tested 63 isolates of *R. secalis* on Igri and 5 other cultivars and reported that Igri which carry BRR4 resistance gene showed resistant reaction to the majority of 46 isolates. Moreover, in Syria, Arabi et al. (2009) assessed 115 isolates of scald against 10 barley differential genotypes. They found Igri and Tadmor cultivars as the most resistant genotypes. In another study in Iran, Beigi et al. (2011) studied the reactions of 47 isolates of *R. secalis* on 8 barley scald differential genotypes. They found Igri and Armelle cultivars as the most resistant cultivars. In their study, cultivar Digger was found as the most susceptible cultivar.

In our current study, out of 30 pathotypes, Modoc and Bey cultivars were found resistant to 19 and 23 pathotypes, respectively. Xi et al. (2002) in Canada found that Modoc and Kitchin cultivars were resistant to 97.7% and 87.1% of isolates of *R. secalis*, respectively. In another study carried out by Abang et al. (2006), Modoc and Bey cultivars were found as the most resistant cultivars among barley genotypes to scald disease. Bouajila et al. (2006) recognized Modoc and Bey cultivars as resistant and moderately resistant with 60% and 50.5% resistance to R. *secalis*, respectively. In a study reported by Takeuchi and Fukuyama (2009), Modoc and Bey cultivars with resistance to 50 pathotypes (86.2% pathotypes) were reported as the second most resistant cultivars after the Osiris cultivar.

In our research, Athene cultivar exhibited a susceptible reaction to 36 (69%) of the isolates. Abang et al. (2006) reported that the Athene cultivar showed 62.5% susceptibility. In another study, the Athene cultivar was susceptible to all isolates tested (Bouajila et al. 2010).

In our study, barley differential cultivars Pirate and Trebi showed resistant reactions to 20 (67%) and 21 (70%) out of 30 (100%) *R. commune* pathotypes, respectively. Bouajila et al. (2006) found resistant and moderately resistant reactions in Pirate and Trebi against scald by having resistance to 76% and 50.5% of pathotypes. In another study, Pirate and Trebi cultivars exhibited resistant and moderately resistant reactions to 63.89% and 58.34% of the scald pathotypes, respectively (Bouajila et al. 2010). In Canada, in a study conducted by Xi et al. (2002), Trebi cultivar showed resistant reaction against 94.5% of the scald pathotypes. Abang et al. (2006) reported that cultivar Pirate exhibited a resistant reaction to all scald isolates and cultivar Trebi exhibited a resistant reaction to 87.5% of scald isolates.

Bülbül 89 and Efes 3 cultivars were used in our study as the susceptible control cultivars. Fifty (96%) *R. commune* isolates showed virulent reactions on these two cultivars. Although these two susceptible control cultivars in this study and in the other recent studies (Azamparsa et al. 2015a, 2015b) were found to be susceptible to *R. commune*, resistance of these two control cultivars against some isolates were observed in this study, as well. Among 52 isolates just two isolates, Sivas-Gemerek (GPS 31) and Mardin-Midyat (13-147), produced limited symptoms and these cultivars were placed in the resistant group. These two susceptible cultivars were not included in pathotype groups assessment and they were excluded in pathotype categories. These two susceptible cultivars, Efes 3 and Bülbül 89 showed mean disease reactions of 3.8 and 3.45 out of 4, respectively. Mert and Karakaya (2004) obtained disease scale values of 3.7 and 4 for Bülbül 89 and Efes 3 cultivars, respectively. Bülbül 89 and Efes 3 cultivars showed resistant reactions as compared to two barley scald differential cultivars Athene and Digger when inoculated with isolate of Mardin-Midyat (13-147) of *R. commune*. These results showed that at least one resistance gene or factor might be present in susceptible control cultivars Efes 3 and Bülbül 89. Among the barley differential cultivars, Jet and Abyssinia showed the most resistant reactions to *R. commune* and these two differential cultivars may be used as genitors in future barley scald resistance breeding programs. Also, wild barley (*Hordeum spontaneum*) and barley landraces can be used in scald resistance breeding studies (Azamparsa et al. 2019). For better management of the disease, information about the pathotype composition of fungus is necessary. In breeding studies pathotypes of the fungus should be considered.

Information about the pathotype composition of fungus is necessary for managing scald disease of barley. In this study, 52 *R. commune* single spore isolates were obtained from different regions of Turkey and 30 scald pathotypes were determined based on virulence on 17 barley scald differential cultivars. Eighteen of these pathotypes were from Central Anatolia region, 14 pathotypes were from Southeast Anatolia region, 6 pathotypes were from Aegean region, and 1 pathotype was from Black Sea region of Turkey. Twenty, 5, 1, 3 and 1 of these pathotypes were represented by 20, 2, 3, 4 and 7 isolates, respectively. Among the 17 barley scald differential cultivars, Jet and Abyssinia were susceptible to 1 pathotype, on the other hand, cultivar Steudelli was susceptible to 24 pathotypes and cultivar Digger was susceptible to 25 pathotypes. Two susceptible control cultivars Bülbül 89 and Efes 3 were susceptible to 93% of scald pathotypes. It appears that considerable variation exists among the Turkish *R. commune* isolates obtained from some barley growing areas of Turkey.

ACKNOWLEDGEMENTS

This study is part of the Ph.D. thesis of M.R. Azamparsa submitted to Ankara University Graduate School of Natural and Applied Sciences and is financially supported by the Scientific and Technological Research Council of Turkey (Project No 111O644).

ÖZET

Rhynchosporium commune fungal etmeni tarafından meydana getirilen Arpa yaprak lekesi hastalığı, dünyada ve Türkiye'de arpanın önemli bir hastalığıdır. 2012, 2013 ve 2014 yıllarında Türkiye'nin değişik bölgelerinde sürveyler düzenlenmiştir. Sürvey sonucu toplanan örneklerden izolasyonlar yapılmış ve bunlardan 52 tane *R. commune* tek spor izolatı seçilmiştir. On yedi adet arpa ayırıcı test çeşidi üzerindeki virülenslik değerlendirmelerine bağlı olarak toplam 30 arpa yaprak lekesi patotipi belirlenmiştir. Bu patotiplerin 18 adedi Orta Anadolu Bölgesi, 14 tanesi Güneydoğu Anadolu Bölgesi, 6 adedi Ege Bölgesi ve 1 adedi ise Karadeniz Bölgesi sürvey örneklerinden elde edilmiştir. Bu patotiplerin 20, 5, 1, 3 ve 1 adedi 20, 2, 3, 4 ve 7 izolat ile temsil edilmişlerdir. Patotiplerin hiçbiri 17 ayırıcı test çeşidi ve 2 hassas çeşit üzerine virülent olarak belirlenmemiştir. En virülent patotipler (patotipler 29 ve 30), Manisa-Kula (13-203) ve Gaziantep-Subağı (GPS71U) lokasyonlarından toplanan örneklerde, en az virülensliğe sahip patotip ise Sivas-Gemerek (GPS31) ve Sivas-Ulaş (NKT20) lokasyonlarından toplanan örneklerde belirlenmiştir (patotip 1). On yedi arpa ayırıcı test çeşidi içinde Jet ve Abyssinia 1 patotipe, Osiris, Atlas 46 ve Forrajera 3 patotipe, La Mesita ve Bey 7 patotipe, Trebi 9 patotipe, Pirate 10 patotipe, Modoc 11 patotipe, Kitchin ve Igri 12 patotipe, Armelle ve Astrix 19 patotipe, Athene 21 patotipe, Steudelli 24 patotipe ve Digger 25 patotipe hassas reaksiyon göstermişlerdir. Arpa ayırıcı test çeşitleri içinde Jet ve Abyssinia çeşitleri en dayanıklı çeşitler olarak, Digger ve Steudelli çeşitleri ise en hassas çeşitler olarak belirlenmişlerdir. Hassas kontrol çeşitleri Bülbül 89 ve Efes 3, arpa yaprak lekesi patotiplerinin %93'üne hassas reaksiyon göstermişlerdir. Türkiyenin bazı arpa üretim alanlarından elde edilen *R. commune* izolatlarında oldukça fazla varyasyonun olduğu görülmektedir.

Anahtar kelimeler: *Rhynchosporium commune*, ayırıcı set, arpa yaprak lekesi patotipleri, *Hordeum vulgare*

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Cite this article: Azamparsa, M, Karakaya, A. (2020). Determination of the pathotypes of *Rhynchosporium commune* (Zaffarona, McDonald & Linde) in some regions of Turkey. Plant Protection Bulletin, 60-3. DOI: 10.16955/bitkorb.460235

Atıf için: Azamparsa, M, Karakaya, A. (2020). Türkiye'nin bazı bölgelerinde *Rhynchosporium commune* (Zaffarona, McDonald & Linde)'nin patotiplerinin belirlenmesi. Bitki Koruma Bülteni, 60-3. DOI: 10.16955/bitkorb.460235