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First Report of *Fusarium redolens* Causing Root and Crown Rot of Barley (*Hordeum vulgare*) in Turkey

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ABSTRACT

Barley (*Hordeum vulgare* L.) is an important cereal crop in terms of human and animal nutrition in the world. *Fusarium* species are a diverse group of fungi which damage small-grain cereals by rotting the seedlings, roots, crowns and in some cases, infect the heads. In order to determine the root rot diseases, surveys were carried out in barley growing areas in Kırşehir and Kırıkkale provinces of Central Anatolia Region in 2011. As a result of isolation from diseased barley plants, three isolates *Fusarim redolens* Wollenweber were found in Kırşehir Provinces. The identification of fungi to the species level was carried out according to morphology and DNA sequence analysis. Pathogenicity tests were conducted such as hypocotly test and plant test. This is the first report of *Fusarium redolens* isolated from barley plants in Turkey.

Key words: *Fusarium redolens*, barley, root rot

ÖZET

Türkiye'de Arpada Kök ve Kökboğazı Çürüklüğüne Neden Olan *Fusarium redolens*'in İlk Tespiti

Arpa (*Hordeum vulgare* L.), dünyada insan ve hayvan beslenmesi açısından önemli bir hububat bitkisidir. *Fusarium* türleri hububatta fide çürüklüğü, kök ve kökboğazı çürüklüğü ve bazı durumlarda başak yanıklığına neden olarak zarar oluştururlar. Kök çürüklüğü etmenlerinin belirlenmesi amacıyla 2011 yılı yetiştirme sezonunda İç Anadolu Bölgesi'nde yer alan Kırşehir ve Kırıkkale illerindeki arpa yetiştirme alanlarında surveyler yapılmıştır. Hastalıklı arpa bitkilerinden yapılan izolasyonlar sonucunda Kırşehir ilinden toplanan 3 izolatın *Fusarim redolens* olduğu tespit edilmiştir. Tür tanımlamaları morfolojik ve DNA sekans analizi ile gerçekleştirilmiştir. İzolatların patojenisite testleri petride hipokotil testi ve bitki testi ile yapılmıştır. Bu çalışma sonucu tespit edilen *Fusarium redolens* arpada Türkiye için ilk kayıttır.

Anahtar Sözcükler: *Fusarium redolens*, arpa, kök çürüklüğü

INTRODUCTION

Barley (*Hordeum vulgare* L.) is one of the important crops of agriculture, and it is the second most important cereal grain with 6,7 million tone production in Turkey (Anonymous, 2016). It is used as malt in brewing and distilling industry, as an additive for animal feed, and as a component of various food and bread for human consumption. Root and crown rots are important and widespread cereal diseases that are present in most parts of Turkey, including the Central Anatolia Region and root and crown rot is considered a disease complex. Pathogens associated with the complex include *Bipolaris sorokiniana* (Sacc.) Shoemaker (teleomorph *Cochliobolus sativus*

((Ito & Kurib.) Drech. ex Dast.), *Rhizoctonia* spp., *Microdochium nivale* (Fr.:Fr.) Samuels & I.C. Hallett (=*Fusarium nivale* (Fr.) Sorauer), and several *Fusarium* spp. The two most frequently reported *Fusarium* species are *F. pseudograminearum* O'Donnell & Aoki and *F. culmorum* W.G. Smith, while several others such as *F. acuminatum*, *F. avenaceum*, *F. oxysporum*, and *F. crookwellense* have also been reported on cereals in Turkey (Tunalı et al. 2008). *Fusarium* spp are the most important group of fungi which damage to barley by rotting the seed, seedlings, roots, crowns, basal stems, and in some cases, infect the heads or spikes in Turkey.

Fusarium redolens has been first reported to cause crown rot on durum wheat in Saskatchewan, Canada (Taheri 2011) and crown rot of bread and durum wheat in Turkey (Gebremariam et al. 2015) on cereals. It has been also reported rot of onions (Bayraktar and Dolar, 2011; potatoes (Çolakoglu, 1986), tomatoes (Erol, 2007; Özer and Soran, 1991), chickpeas (Tekeoglu et al., 2017), lentils (Özer and Soran, 1991) and beans (Soran, 1981) in Turkey.

MATERIALS and METHODS

Plant collection and isolation

In order to determine the *Fusarium* species and their pathogenicity associated with root and crown rot of barley in Kırşehir and Kırıkkale Provinces, 36 samples of barley were collected in 2011 growing season. Segments of necrosed were surface disinfected in 0,5% (w/v) sodium hypochlorite solution for 1-2 min, rinsed three times in sterile distilled water before they were placed onto potato dextrose agar (PDA, Merck) containing streptomycin. Single spore isolates were obtained from fungal cultures incubated at 23±1°C with a 12-h photoperiod for 2 or 4 days, and stored on PDA slant tubes at 4°C.

Pathogenicity tests

Pathogenicity tests were conducted hypocotyl test with all isolates and also tested on seedlings grown in pots all isolates on susceptible barley cultivar (cv. Kral 97). For hypocotyl tests, isolates were incubated on PDA at 25°C for 2 days; mycelial discs (4mm) from an actively growing edge of the fungal culture were transferred to 2% WA and incubated at the same conditions for 2 days. Seeds of the susceptible barley cultivar Kral 97 were disinfected by dipping in 1% NaOCl for 5 min, blotted dry with sterile paper towels, then six seeds were placed adjacent to the growing edge of the isolates in each Petri dish in sterile conditions. PDA discs were used as controls. Five replicate plates were used for each isolate after incubation for 7–8 days at 25°C, roots and hypocotyls of the plants were examined and evaluated for disease severity (Ichielevich-Auster et al., 1985).

For plant tests, 50 g of sand-maize meal medium (9:1) was prepared and this medium was autoclaved two times in the 200 ml flasks at 121°C for 20 minutes. Each flask was inoculated with 5 disc-of fungus (*F. redolens*) from a pure culture and incubated at 25^0C for 14 days. After incubation, the inoculum (sand maize+fungus) was added 50 gr to one pot containing 600 gr of soil mix (garden soil, washed river gravel, burnt farm manure, 1: 1,5:0,5, v/v/v). After 5-6 days, ten seeds of susceptible cultivar Kral 97 were planted in each plastic pot (13 cm in diameter) in the greenhouse conditions at 20 ± 5 °C. There were four replicate pots for treatment. Uninfested soil was used for control pots. Disease severity of root rot was evaluated on scale of 0 to 5 (Ichielevich-Auster et al. 1985), where: 0= no disease, 1= 1-10%, 2= 11-30%, 3= 31-50%, 4= %51-80, 5= the entire hypocotyl infected. These scale values were converted to disease severity values using the following formula (Karman 1971).

Disease Severity= [∑ (no. of plant in category x category value)] x 100 / Total no. of plants x max. category value).

Identification

Monosporic cultures were identified as *Fusarium redolens* Wollenw. by morphology (Leslie and Summerell 2006) and DNA sequence analysis. The ITS regions of the isolates were amplified using the universal primers ITS-1 (5' TCC GTA GGT GAA CCT GCGG 3') and ITS-4 (5' TCC TCC GCT TAT TGA TATGC 3') as described by White et al. (1990). Genomic DNA was extracted using a Qiagen DNeasy ® Plant Mini Kit, as specified by the manufacturer, and stored at -20 ºC prior to use. PCR reaction mixtures and condition were modified from previous studies (Aroca and Raposo 2007; Cobos and Martin, 2008). The reaction mixtures of PCR, a final volume of 50 µl, contained 5µl of 10X buffer [75 mM Tris HCl, pH 9.0, 50 mM KCl, 20 mM (NH4)2SO4], 2 µl of 5 µM each primers, 5 µl of 1.5mM MgCl2, 2 µl of 10 mM deoxynucleoside triphosphates (dNTPs), 1U Taq polymerase (Fermentas), 5 µl of DNA template for each reaction and 5 µl of bovine serum albumin (BSA:10 mg/ml). DNA amplifications were carried out in a Techne TC-5000 thermal cycler by the following program: 94 ºC for 2 min, followed by 34 cycles of (1) denaturation (94˚C for 30 s), (2) annealing (60˚C for 30 s) and (3) extension (72 ˚C for 30 s), and a final extension step 10 min at 72˚C. The PCR products were separated in 1.5 % agarose gels stained with ethidium bromide and visualized under UV light. They were sequenced by REFGEN (Gene Research and Biotechnology Company, Ankara, Turkey). The nucleotide sequences were subjected to Basic Alignment Search Tool (BLAST) analysis (http://www.ncbi.nlm.nih.gov) and compared to other sequences in GenBank.

RESULTS and DISCUSSION

In the surveys, 106 barley fields were examined and 36 diseased barley samples were collected in Kırşehir and Kırıkkale Provinces. In consequence of isolations, three *F. redolens* isolates were obtained in Kırşehir Provinces. Regarding the pathogenicity test on agar plates, all three *F. redolens* isolates were found to be pathogenic (56,67-76,67%) on susceptible barley cultivar Kral 97. All isolates tested in plastic pots in greenhouse conditions and average disease severity value, was found to be % 64,00-80,67. *F. redolens* isolates caused significant reduction of emergence, stunting, reduction in the number of seminal roots and superficial discolouration on the hypocotyls and roots on barley. Non-inoculated plants remained healthy. The pathogen was reisolated from diseased plants.

Morphologic identifications were performed according to Leslie and Summerell (2006). The culture development of the effect PDA medium was white to pink with some brown pigmentation in the agar. The colony diameter reached 75-80 mm on PDA in 10 days at 23 ± 1ºC (Figure 1 a). The upper cells of macroconidia were wider, the end cell was hook shaped and the base cells were in the shape of a foot. Macroconidia were 3-5 septa and 45.9 × 2.58 µm in size (Figure 2 a). The chlamydospore formation was usually observed quite abundant especially as the culture ages and they were spherical to oval shape, rough walled, and slightly pigmented. The monofilaments bearing microconidia on hyphae were short in size (Figure 1 b). The microconidia were oval or cylindrically shaped or 1 parted and have dimensions of 10,68 × 3,18 µm (Figure 2 b).

F. redolens has been described for the first time by Wollenweber in 1913. It is a relatively common soil-borne fungus found in temperate areas. It has been recorded on a variety of hosts associated with root rot diseases (Leslie and Summerell 2006) including cereals and vegetables. The fungus has been reported as pathogen on wheat in Ankara, potato in Erzurum, tomato in Izmir and Samsun, bean in Adana, lentil in Ankara, onion bulbs in Ankara, Eskisehir, Yozgat and Amasya, chickpea in Kutahya, Denizli, Burdur, Isparta, Konya, Sivas, Yozgat, Corum, Antalya and Samsun Provinces in Turkey (Gebremariam et al. 2015; Bayraktar and Dolar, 2011; Çolakoglu, 1986; Erol, 2007; Özer and Soran, 1991;Tekeoglu et al., 2017; Özer and Soran, 1991; Soran, 1981).

Fusarium root and crown rot is one of the main causal agents of dryland root rot on barley in Turkey. In present study, the three pathogen *Fusarium* isolates obtained from barley were identified as *F. redolens*. Results of this study present the first report of *F. redolens*, causing disease on barley in Turkey. Because of wide host range and in suitable climate conditions, it can cause severe yield losses. *F. redolens* is a threat to barley growing areas.

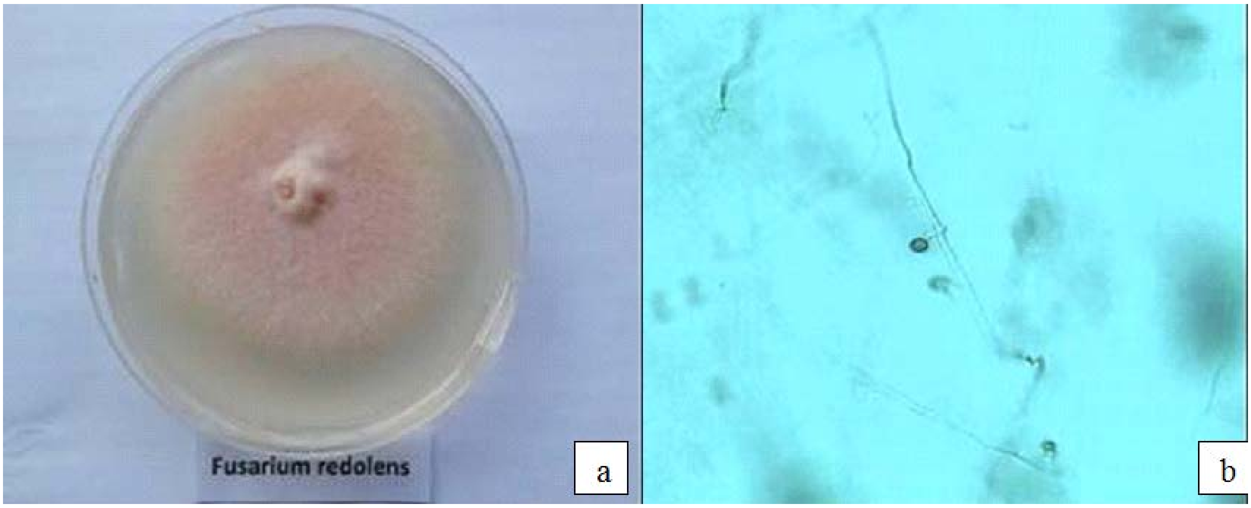

Figure 1. a. *Fusarium redolens* colony development on PDA medium (10 days), b. Microconidia heads on monophtalites (× 10 µm) under the light microscope.

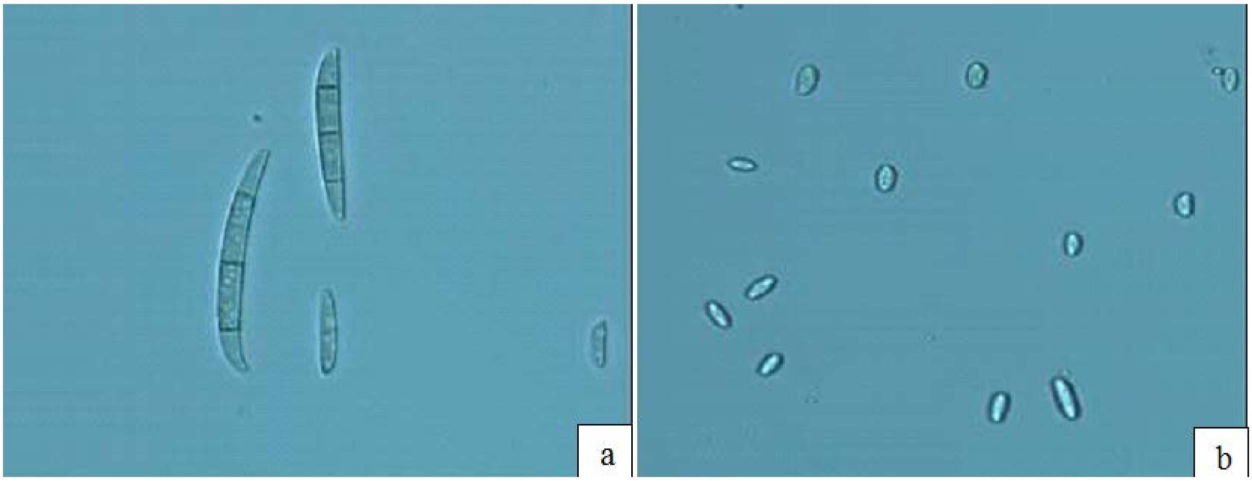

Figure 2. Appearance under the microscope of *Fusarium redolens,* a. Macroconidia of *Fusarium redolens* (× 40µm), b. Microconidia of *Fusarium redolens* (× 40 µm).

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