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FIRST REPORT OF *RHIZOCTONIA ZEAE* CAUSING STUNTING AND ROOT ROT ON WHEAT IN TURKEY

Filiz ÜNAL[1*], F. Sara DOLAR[2], Süreyya ÖZBEN[1]

[1]Ministry of Agriculture and Rural Affairs, Central Plant Protection Research Institute, Ankara, Turkey
[2]Department of Plant Protection, Faculty of Agriculture, Ankara University, Ankara, Turkey
*(Corresponding author: unalflz06@gmail.com)

Abstract

Rhizoctonia is a destructive soilborne pathogen with a wide host range in the world. It is one of the main causal agents of dryland root rot on wheat in Turkey. Wheat is widely planted in the Central Anatolia Region in Turkey. In order to identify species of *Rhizoctonia*, surveys of wheat fields in the Konya, Ankara, Eskisehir, Yozgat and Kırıkkale (provinces in the Central Anatolia Regions) were undertaken. Three of the *Rhizoctonia* isolates collected from necrotic lesions on the root and crown were identified as *Rhizoctonia zeae* (teleomorph: *Waitea circinata* var. *zeae*), as well four of the isolates from rhizosphere soils. Species identification were done according to the basis of hyphal and colony morphology, anastomosis reaction with known tester isolates and comparing sequences of Internal Transcribed Spacer (ITS) region. Colonies growth on PDA were orange when young and became salmon colored with age. Sclerotia were uniform and nearly spherical, mostly 0.2 to 0.5 mm in diameter, initially orange and turned brown during time. The number of nucleus in each hypha cell was 4 to 8. The resulting sequences were compared to other sequences and were 82 to 95% identical to other *R. zeae* sequences in GenBank.

First pathogenicity test was conducted with agar- plate assay with all isolates and then it was tested on seedlings grown in pots the most virulent isolate on susceptible wheat cultivar. Test was done in the greenhouse conditions at 23 ± 2 °C, with a 12-h photoperiod and 50–60% RH. Average disease severity value was determined as 81%. Pathogenicity tests revealed that *Rhizoctonia zeae* caused significant reduction of emergence, stunting, reduction in the number of seminal roots and superficial discolouration on the hypocotyls and roots on wheat. Non-inoculated plants remained healthy.

This is the first report of *R. zeae* isolated from wheat plants and rhizosphere soils in Turkey

Keywords: Rhizoctonia zeae, first report wheat, Turkey

Introduction

Rhizoctonia is a destructive soilborne pathogen with a wide host range in the world. It is one of the main causal agents of dryland root rot on wheat in Turkey (Tunalı *et al*., 2008). Wheat is widely planted in the Central Anatolia Region that included the Konya, Ankara, Eskisehir, Yozgat and Kırıkkale provinces. *Rhizoctonia zeae*, the anamorph of *Waitea circinata* var. *zeae*, was initialy described as a corn pathogen in USA, India, Argentina and China (Voorhees, 1934; Sumner and Bell 1982; Gunnell, 1986; Li *et al.*, 1998). Later, this fungus was also reported to cause disease on other important crops such as sugarbeet, wheat, bean, soybean and turfgrasses (Kuznia and Windels 1994; Tredway and Burpee 2001; Erper *et al*., 2005).

R. zeae is the causal agent of reduced total emergences and stands of wheat (Kuznia and Windels, 1994). Although this fungus was previously reported in Turkey from Johnsongrass (Demirci and Eken, 1999), corn kernels (Demirci and Kordali, 1999), bean and soybean roots (Erper *et al.*, 2005), this is the first report of *R. zeae* isolated from wheat plants and rhizosphere soils.

Materials and Methods

Plant collection and isolation

In order to determine the anastomosis groups and pathogenicity of *Rhizoctonia* species associated with stunting, bare patch, root and crown rot of wheat in Turkey, 330 samples of wheat and rhizosphere soil were collected in 2009 and 2010 growing seasons. Samples were taken from fields in 58 districts of Konya, Ankara, Yozgat, Eskişehir and Kırıkkale provinces. Segments of necrosed tissue were placed on acidified water agar (1.5 % water agar amended 3 ml of 10 % lactic acid per liter of medium). Hyphal tips of *Rhizoctonia*-like fungi were transferred to Potato Dextrose Agar (PDA, Merck, Germany) containing 50 mg/l streptomycin sulfate. Sterile wheat straws were used for *Rhizoctonia* spp. isolation from soil samples. Soil samples (collected with plants) from the respective fields were transferred to pots on a greenhouse bench (20 ± 2^0C). Plots were then watered to field capacity.About 4 cm long internodal segments of mature, dried wheat straw were inserted vertically, 4 per pot, and left for 3 or 4 days After that straws were removed, washed, blotted and placed on acidified water agar. Isolates of *Rhizoctonia* were transferred to PDA (Ogoshi *et al.*, 1990).

Identification

In order to determine hyphal diameter and the number of nuclei per cell of the isolates, Rhizoctonia isolates were maintained on PDA in an incubator regulated at 25 °C and in darkness. Developing mycelia were stained with safranin O (Sigma, USA) and 3% KOH (Bandoni, 1979) and observed under phase contrast microscopy at x 400 magnification.. Hyphal diameter was determined by measuring 10 cells. Nuclei were counted in 15 cells.
Anastomosis was tested by pairing isolates with representative testers of *Waitea* spp. *Rhizoctonia* isolates and *Waitea* tester isolates (Rz 590: *R. zeae*; Ro 231: *R. oryzae*, W 616: *Waitea circinata* var. *circinata*) were activated on PDA at 25 °C in the dark. Coverslips, sterilized by dipping in 95 % ethyl alcohol and flaming, were coated with a thin layer of 0.5 % PDA and placed on water agar plates. Agar plugs with mycelia of *Rhizoctonia* isolates and the tester isolates were cut the margin of a growing colony and transfered to water agar plates on the opposite sides of the coverslip. After incubation at 25 °C for 24-48 h in the dark, when overlapping mycelia of two isolates were observed, the coverslip was removed from the plate and placed on a slide in the mixture of one drop of safranin O and one drop of 3 % KOH. Stained hyphae were observed microscopically . Anastomosing hyphae were traced back to their source in order to confirm the anastomosis between our isolates and the tester isolates (Kronland and Stanghellini, 1988). For the anastomosis testing, all pairs were examined twice.

Pathogenicity tests

First pathogenicity test was conducted with agar- plate assay with all isolates. Pathogenicity was also tested on seedlings grown in pots the most virulent isolate on susceptible wheat cultivar (cv. Sultan 95). Seedlings were grown in plastic pots (5 cm in diameter) in the greenhouse conditions at 23 ± 2 °C with a 12-h photoperiod and 50–60 % RH. Inoculum was prepared on the moistened sterile wheat grains in tests tubes. The bottom of the pots were filled with 40 cm^3 of medium vermiculite and topped with 30 cm^3 of natural sterile silt loam. Eight pathogen-colonized wheat grains were placed in the middle of the soil layer. Control was consisted of pots without inoculum.
All pots were covered with clear polyethylene and incubated 5 days. There were six replicate pots for treatment. After 5 days, eight seeds of wheat were placed on the soil surface, covered with 10 cm^3 of steril natural topsoil, and watered with 10 ml of distilled water. After 3 weeks, the plants

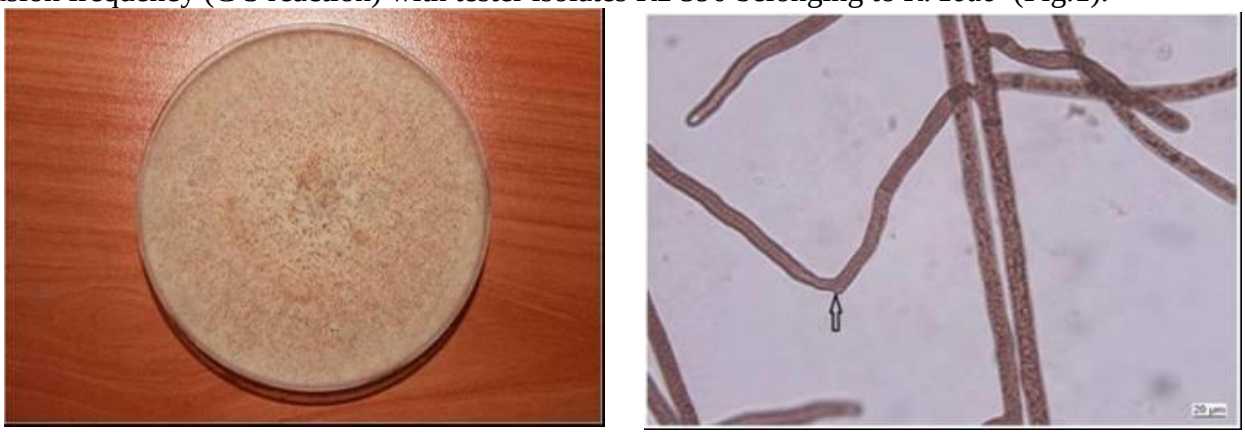
were washed free of soil (Paulitz *et al*., 2003). Severity of *Rhizoctonia* root rot was evaluated on a scale of 0 to 8 (Kim *et al.*, 1997).

Molecular Analysis

Approximately, 300 mg mycelium were harvested and ground with liquid nitrogen in a sterile mortar for DNA extraction from culture medium. Genomic DNA was extracted using a Qiagen DNeasy ® Plant Mini Kit, as specified by the manufacturer, and stored at -20 ^{0}C 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 5 µl 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 $MgCl_2$, 2 µl of 10 mM deoxynucleoside triphosphates (dNTPs), 1 U Taq polymerase (Fermatas), 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 C for 2 min, followed by 34 cycles of (1) denaturation (94˚C for 30 s), (2) annealing (60˚C for 30 s) and (3) extention (72 ˚C for 30 s), and a final extension step 10 min at 72 ˚C.
The ITS region of the isolate was 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’). 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).

Results and Discussion

The nucleus number that was found in each hypha cell was 4-8, and width of the main runner hyphae was more than 7µm. According to the cellular nucleus number, width of the main runner hyphae, colony morphology and the anastomosis test, seven isolates were identified as *R. zeae.* As a result of anastomosis test with testers isolates of *Waitea* spp, these isolates anastomosed with high fusion frequency (C 3 reaction) with tester isolates Rz 590 belonging to *R. zeae* (Fig.1).



a b

Figure 1. 28-day old colony appearance of *Rhizoctonia zeae* on potato dextrose agar (a); anastomosis between hyphae (b)

Morphological features of isolates on PDA were similar with descriptions of Demirci (1998) and Telmadarrehei *et al*.(2011). Colonies were orange when young, turning salmon coloured with age. Sclerotia were uniform and nearly spherical, mostly 0.2 to 0.5 mm in diameter, initially orange and turned brown during time .

The resulting sequences were compared to other Rhizoctonia sequences and were 82 to 95% identical to other *R. zeae* sequences in the GenBank. They were submitted to the GenBank with the accession numbers of KC590513, KC590514, KC590515, KC590516, KC590517, KC590518 and KC590587.

Regarding the pathogenicity test on agar plates, all seven *R. zeae* isolates were found to be pathogenic (72- 98%) on susceptible wheat cultivar. The most virulent isolate tested in plastic pots in greenhouse conditions and average disease severity value, was found to be 81%. *R. zeae* isolate caused significant reduction of emergence, stunting, reduction in the number of seminal roots and superficial discolouration on the hypocotyls and roots on wheat. Non-inoculated plants remained healthy. The pathogen was reisolated from diseased plants.

Rhizoctonia zeae was described for the first time in 1934 in Florida (USA) as causing agent of ear rot of corn (*Zea mays* L.) (Voorhees, 1934). The fungus has also been reported on the other important crops, such as wheat, alhough there are few publications related to *R. zeae* on wheat in the world (Kuznia and Windels 1994; Telmadarrehei *et al*.., 2011). *R. zeae* is the causal agent of reduced total emergences, stands and eye spot of wheat in USA and Iran (Kuznia and Windels 1994; Telmadarrehei *et al*., 2011). Result of pathogenicity test presented in this study, similarly as results of Kuznia and Windels (1994), showed that *Rhizoctonia zeae* caused significant reduction of emergence, stunting, reduction in the number of seminal roots and superficial discolouration on the hypocotyls and roots on wheat.

Conclusion

Rhizoctonia is one of the main causal agents of dryland root rot on wheat in Turkey. In present study the *Rhizoctonia* isolates, obtained from wheat, were identified as *R. zeae*. It is known that the host range and diseases caused by the isolates from different species or anastomosis group are different. Therefore, determination of the species or anastomosis grouping of the isolates is very important. Results of this study present the first report of *R. zeae*, causing disease of wheat in Turkey. Due to the severe symptoms, it should be considered a potential threat to wheat cultivation in Turkey.

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References

Aroca, A., Raposo, R**.** 2007. PCR-based stragety to detect and identify species of Phaeoacremonium causing grapevine diseases. *Applied and Environmental Microbiology*, 73, 2911-2918.

Bandoni, R.J. 1979. Safranin O as a rapid strain for fungi. *Mycologia,* 71, 873-874.

Cobos, R., Martin, M.T. 2008. Molecular characterisation of *Phaeomoniella chlamydospora* isolated from grapevines in Castilla y León (Spain). *Phytopathol. Mediterr.*, 47, 20–27.

Demirci, E. 1998. *Rhizoctonia* species and anastomosis groups isolated from barley and wheat in Erzurum, Turkey. *Plant Pathology*, 47(1): 10-15.

Demirci, E., Eken C. 1999. First Report of *Rhizoctonia zeae* in Turkey *Plant Disease* 83, 200.

Demirci, E., Kordali S. 1999. *Rhizoctonia* species and anastomosis groups from corn kernels in Turkey. *Plant Disease,* 3(9), 879.

Erper, İ., Karaca, G., Özkoç, İ. 2005. First Report of Root Rot of Bean and Soybean Caused by *Rhizoctonia zeae* in Turkey. *Plant Disease*, 89, 203.

Gunnell, P.S. 1986. Characterization of the telemorphs of *Rhizoctonia oryzae-sativae, Rhizoctonia oryzae* and *Rhizoctonia zeae* and the effect of cultural practices on aggregate sheath spot of rice, caused by *Rhizoctnia oryzae sativae*. Ph.D. thesis, University of California, Davis.

Kim, D.S., Cook, R. J., Weller, D.M. 1997. *Bacillus* sp. L324-92 for biological control of three root diseases of wheat grown with reduced tillage. *Phytopathology*, 87: 551-558.

Kronland, W.C., Stanghellini, M.E. 1988. Clean slide technique for the observation of anastomosis and nuclear condition of *Rhizoctonia solani*. *Phytopathology,* 78, 820-822.

Kuznia, R.A.,Windels, C.E. 1994. *Rhizoctonia zeae* pathogenic to spring wheat and
sugarbeet seedlings. *Phytopathology*, 84, 1159.

Li, H.R., Wu, B.C., Yan, S.Q. 1998. Aetiology of *Rhizoctonia* in sheath blight of maize in Sichuan. *Plant Pathology*, 47: 6-21.

Ogoshi, A., Cook, R.J., Bassett, E.N. 1990. *Rhizoctonia* species and anastomosis groups causing root rot of wheat and barley in the Pacific Northwest. *Phytopathology,* 80(9),785-788.

Paulitz, T.C., Smith J.D., Kidwell, K.K. 2003. Virulence of *Rhizoctonia oryzae* on wheat and
barley cultuvars from the Pacific Northwest. *Plant Disease,* 87(1), 51-55.

Sumner, D.R., Bell D.K. 1982. Root diseases induced in corn by *Rhizoctonia solani* and
Rhizoctonia zeae. *Phytopathology*, 72:86–91.

Telmadarrehei, T., Tajick Ghanbary, M. A., Rahimian, H., Rezazadeh, A., Javadi, M.A. 2011. Isolation and some pathologic properties of *Rhizoctonia zeae* from cultural soils of Golestan and Mazandaran Provinces, Iran. World Applied Sciences Journal 14(3): 374-377.

Tredway, L.P., Burpee, L.L. 2001. *Rhizoctonia* diseases of turfgrass. *The Plant Health Instructor*, DOI: 10.1094/PHI-I-2001-1109-01.

Tunalı, B., Nicole, .J.M., Hodson, D., Uçkun, Z., Büyük, O., Erdurmuş, D., Hekimhan, H.,
Aktaş, H., Akbudak, M.A., Bağcı, S.A. 2008. Root and crown root fungi associated with spring, facultative, and winter wheat in Turkey. *Plant Disease*, 92: 1299-1306.

Voorhees, R.K. 1934. Sclerotial rot of corn caused by *Rhizoctonia zeae*, n. sp. *Phytopathology,* 24, 1290-1303.