Rhizoctonia fragariae causes black root rot on strawberry seedlings in Turkey

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Abstract

Samples of strawberry transplants were randomly collected from 47 growers in Aydın province of Turkey. A total of 10 *Rhizoctonia* isolates were identified by morphological characterization, analysis of rDNA-ITS sequences. Pathogenicity tests were carried on strawberry seedlings and detached stolon. Ten pathogenic binucleate *Rhizoctonia* isolates were defined as AG-A, AG-G and AG-K on the basis of ITS region sequence analysis. To our knowledge, this is the first report of the presence of *Rhizoctonia fragariae* in strawberry transplants in Turkey.

Keywords Black root rot · *Fragaria ananassa* · Binucleate · AG-A · AG-G · AG-K

Strawberry is an economically important fruit that is produced and exported in Turkey. Aydın Province has significant production areas for strawberry in Turkey. The present study was conducted to determine the presence of fungal pathogens in strawberry transplants used by farmers in Sultanhisar and Köşk districts of Aydın province. For this purpose, transplant samples were collected from strawberry production areas from July to August during 2009–2010 and 2010–2011 growing seasons. The fragments from the root and crown of seedlings were aseptically excised after surface disinfection with 1 and 2% sodium hypochlorite (NaOCl), respectively. Potato Dextrose Agar (PDA) plates were inoculated with tissue fragments and incubated at 24 °C for 2–3 days. As a result of macroscopic and microscopic examinations, *Rhizoctonia*-like colonies that show typical hyphal branching according to Sneh et al. (1998) were purified in PDA.

Pathogenicity tests of 10 isolates obtained were performed in strawberry plants (cv. Festival) (Martin 2000) and stolons of strawberry (cv. Festival) (Yildiz and Benlioglu 2014). For pathogenicity tests on strawberry plants, inoculum was prepared by inoculating a mixture of oat bran (67 g), vermiculite (67 g), and water (40 ml) with a 4 mm disk from the edges of seven-day-old PDA cultures of *Rhizoctonia* spp. After 2 weeks incubation at 25 °C, developing inoculum was mixed into soil at the rate of 0.75% (weight/weight). Four-week-old healthy strawberry seedlings (3 seedlings for each isolate) were planted in pots containing inoculum. Uninoculated oat bran, vermiculite mixture was used as a control. The strawberry plants were then placed in a growth chamber under 16-h light/8-h dark conditions at 24 ± 2 °C (Martin 2000). Plants were harvested 5 weeks later and examined for the presence of root and crown rot. All isolates except 1C-35KRh caused root and crown rot on strawberry plants and brown necrotic lesions on detached stolons. However, the isolate 1C-35KRh caused only slight necrotic lesions about 2.3 mm in length on stolons. Control plants remained healthy and asymptomatic. To fulfill Koch's postulates, reisolations were made by plating 5 mm fragment of symptomatic crown and root on PDA and identified as previously described.

Considering that *Rhizoctonia* spp. isolates recovered from diseased strawberry plants were predominantly binucleate (BNR) as previously reported in the USA (Martin 1988, 2000), Israel (Sharon et al. 2007), South Africa (Botha et al. 2003), Italy (Manici and Bonora 2007) and Australia (Fang et al. 2013), we determined the number of nuclei of 10 *Rhizoctonia* spp. isolates. All pathogenic isolates were found

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binucleate after examination under fluorescence microscope by using fluorogenic dye diamidino-2-phenylindole (Martin 1988).

For molecular identification, genomic DNA was extracted from binucleate *Rhizoctonia* spp. isolates by the method of Ceniz (1992). PCR was conducted with ITS1/ITS4 universal primers (White et al. 1990) and the products were sequenced commercially by Macrogen Europe (Amsterdam, Netherlands). The ITS1–5.8S rDNA-ITS2 sequences of the 10 BNR isolates derived from this study were deposited in GenBank with accession numbers of MG547903 to MG547912 (Fig. 1). According to ITS sequence analysis, our ten binucleate *Rhizoctonia* isolates were identified to belong to 3 different anastomosis groups (AG-A, AG-G, and AG-K). In general, 10 Turkish BNR isolates from strawberry in the phylogenetic tree shared 92 to 100% sequence identity among the different AG groups. There was 94 to 100% sequence identity within the group of AG-A, 99–100% within the group of AG-K and 92 to 100% within the group of AG-G.

By comparing the sequences (represented by their DNA accession number) available in GenBank, a maximum likelihood (ML) tree of binucleate *Rhizoctonia* spp. (*Ceratobasidium* spp.) anastomosis groups isolates that are pathogenic to strawberry (Manici and Bonora 2007; Sharon et al. 2007; Fang et al. 2013) was constructed with Mega 7.0 software (Kumar et al. 2016). The ML tree (Fig. 1) based on ITS region sequence data revealed that the 10 BNR isolates were most closely matched to sequences from isolates of AG-A, AG-K and AG-G. Our 4 AG-A isolates clustered into the reference isolates known to be AG-A from strawberry in the USA, China, Israel and Australia with bootstrap support 87% by ML. Our five isolates belonged to AG-G clustered into a distinct clade along with AG-G reference isolate from strawberry in Israel, USA and Italy with bootstrap support of 90% by ML. This suggested these 5 isolates belong to AG-G. The remaining one isolate (MG547912) clustered into a clade containing AG-K reference isolates from Israel and Australia with bootstrap support of 88%.

Fig. 1 The evolutionary history was inferred by using the Maximum Likelihood method based on the Tamura-Nei model (Tamura and Nei 1993) from a ClustalW sequence alignment of rDNA-internal transcribed spacer (ITS) sequences of *R. fragariae* isolates from Turkey and other binucleate *Rhizoctonia* spp. (*Ceratobasidium* spp.) anastomosis groups (AGs) isolates from strawberry available in GenBank. The tree was constructed with Mega 7.0 software (Kumar et al. 2016) with 1000 bootstrap replications. Bootstrap values are positioned alongside the branches with values over 60. Isolate AY684917 [*Athelia* (*Sclerotium*) *rolfsii*] was used as an outgroup. Dots with different color on the left side of accession numbers represent the different anastomosis groups; red AG-A, blue AG-K and Green AG-G

All of 10 *R. fragariae* isolates (4C/68KRh, 14/18KRh, 1/14KRh, 4/61KRh, 3/3KRh, 4/4TRh, 5/1KRh, 1C/48KRh, 20C/52KRh and 1C/35KRh) were stored in the culture collection of the Plant Protection Department, Agricultural Faculty of Adnan Menderes University, Aydin, Turkey. To the best of our knowledge, the present study is the first record for identification of *Rhizoctonia fragariae* in strawberry seedlings in Turkey. These findings are very important for the nurseries, certification procedures and lastly strawberry growers in Turkey.

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