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Rhizoctonia solani AG4 associated with foliar blight symptoms on barley in Iran

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Abstract Since June 2011, foliar blight symptoms have been observed in barley fields at Chaharmahal and Bakhtiari, Iran. The symptoms include water soaked spots progressing to elongated stripe and scald like lesions on the leaves and sheaths of barley plants. From cultural and morphological studies and phylogenetic analysis of sequences of the internal transcribed spacer region (ITS) of the ribosomal DNA, the causal agent was identified as *Rhizoctonia solani* AG4. Pathogenicity tests revealed that *R. solani* AG4 is virulent to barley. To the best of our knowledge this is the first report of *R. solani* AG4 causing foliar disease of barley in Iran.

Keywords Pathogenicity test · Molecular identification · Phylogeny · Sequencing · Cultures · Fungal morphology

Barley (*Hordeum vulgare*) is one of the most important cereal grains in Iran with long historical use as a source of food with high nutritional value. In 2011, approx. 2.5 million tons of barley were produced in Iran (FAO 2011). Generally, the crop yield can be affected by several fungal diseases such as smuts, rusts, leaf scald and mildew. Soil-borne pathogens such as *Rhizoctonia solani* [teleomorph: *Thanatephorus cucumeris*] are also known to cause root and crown rot in barley. *Rhizoctonia solani*, a multinucleate *Rhizoctonia* spp. is distributed nationwide and causes economic damage to a wide range of crops and ornamental plants. The symptoms are varied based on environmental conditions, host and growth phase in which the plants become infected, but the most usual symptoms include damping-off or rot and canker on roots and crowns (Erper et al. 2016; Ogoshi 1987; Sneh et al. 1996). However, it can be found on aerial parts of some herbs, ornamentals and several crops (Bai et al. 2015; Garibaldi et al. 2013; Garibaldi et al. 2015; Holcomb and Carling 2002; Valentín Torres et al. 2016). Although many reports of *R. solani* on various hosts exist in Iran, foliar blight on barley has not been reported prior to this publication.

In June and July 2011, foliar blight symptoms were observed in barley fields at Chaharmahal and Bakhtiari, Iran. Symptoms initially developed as small water-soaked spots with light to dark brown margins starting from stems and progressing rapidly as long stripe and scald like lesions on the leaves and sheaths (Fig. 1). No symptoms were seen on the roots. This research was designed (i) to identify the causal agent/s associated with these foliar blight symptoms and (ii) to investigate the virulence of isolated agent/s using pathogenicity tests.

Representative samples exhibiting foliar blight symptoms were collected from barley fields at Chaharmahal and Bakhtiari. Small pieces from the margin of infected tissues were sterilised in 0.5% sodium hypochlorite (NaOCl) solution for 1 min, followed by washing twice with sterile distilled water, and were then placed on potato dextrose agar (PDA) and incubated at 25 °C for 7 days. Hyphal tips were subcultured on fresh PDA media to assess morphological properties. All isolates formed white to grey colonies on PDA at 25 °C (Fig. 2a). The colonies turned to brown with age and produced monilioid cells but no sclerotia. Mycelia were 3-5 μm in diameter, branched at right angles with a constriction and septum near the branch origin (Fig. 2b, c). Given these morphological and cultural characteristics, the fungal pathogen was identified as *R. solani* (Sneh et al. 1991).

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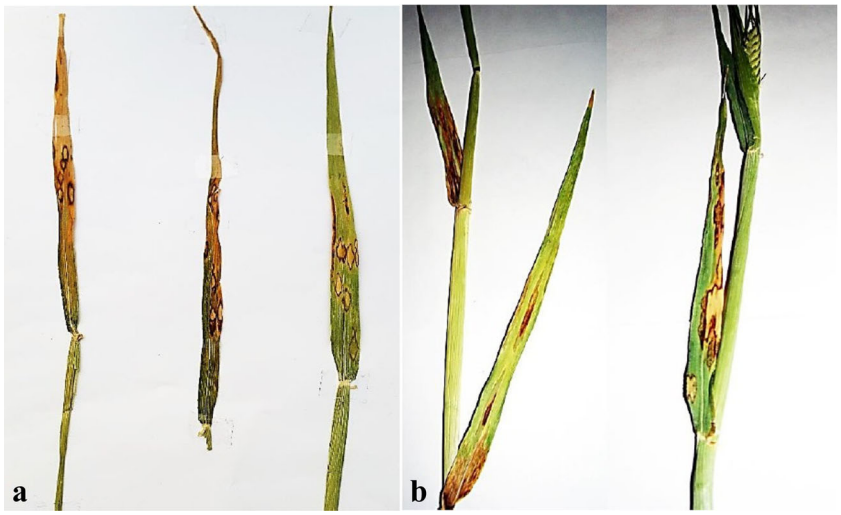

Fig. 1 Foliar blight symptoms on barley: **a** Water soaked spots; **b** Stripe and elongated scald like lesions

Staining the hyphal cells, using 1% safranin O and 3% KOH solution (Bandoni 1979), indicated that the cells were multinucleate (3–6 nuclei). An anastomosis interaction assay was assessed via hyphal fusion interactions with standard anastomosis groups (Kronland and Stanghellini 1988). The isolates were anastomosed with several AG4 tester isolates provided from the Fungal Collection, Department of Plant Protection, Isfahan University of Technology, Isfahan, Iran. In vitro possibility of hymenia formation was evaluated by transferring the fungus from 5-day-old enriched medium (potato dextrose malt agar) incubated in darkness at 26 °C to a poor medium (water agar) as a special sporulation medium. The plates incubated at 26 °C in darkness for 8 days and then were exposed to 12 h photoperiod for 10 days (Murray 1982). No teleomorph formation occurred in any of three repeats of the process.

To confirm morphological identification, molecular characterization was also employed. DNA was extracted using 3% CTAB extraction buffer (Murray and Thompson 1980) and ITS1/4 primers were utilised for ITS rDNA amplification (White et al. 1990). PCR reactions were performed in 15 μl volume containing 15 ng of genomic DNA, 1× Ampliqon *Taq* DNA Pol. 2× Master mix Red, and 0.4 μM of each Primer. A Techne TC-512 thermocycler was used with the following conditions: initial denaturation of 3 min at 94 °C, 30 cycles of 1 min at 94 °C, annealing at 59 °C for 1 min and 2 min at 72 °C with a final extension of 10 min at 72 °C. Amplified fragments were sequenced and the sequence from isolate LO was deposited in GenBank (accession number KX646540). Our sequences had 99% similarity with the reference sequence of *R. solani* AG4 (KC590533.1).

The sequences alignment was performed by ClustalW algorithm and a phylogenetic tree was constructed based on the Neighbor-Joining statistical method (NJ) with 1000 bootstrap replicates using MEGA6 software (Tamura et al. 2013). *Tulasnella calospora* was used as the out-group. Phylogenetic analysis of isolate LO with other *Rhizoctonia* spp. revealed that the

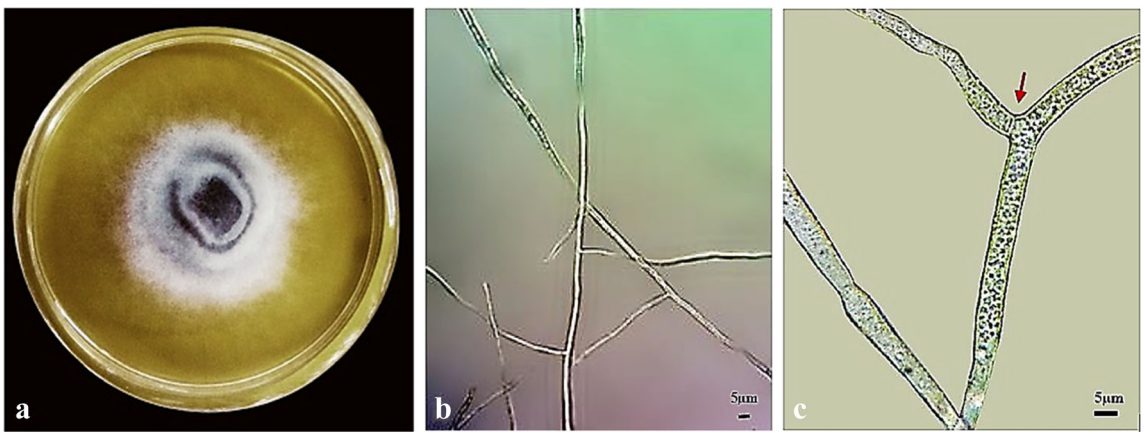

Fig. 2 **a** Colony of *Rhizoctonia solani* from isolate LO on PDA, **b, c** Microscopic Mycelia of *Rhizoctonia solani* (Right-angled branching and constriction at the base of hyphal branches of isolate LO)

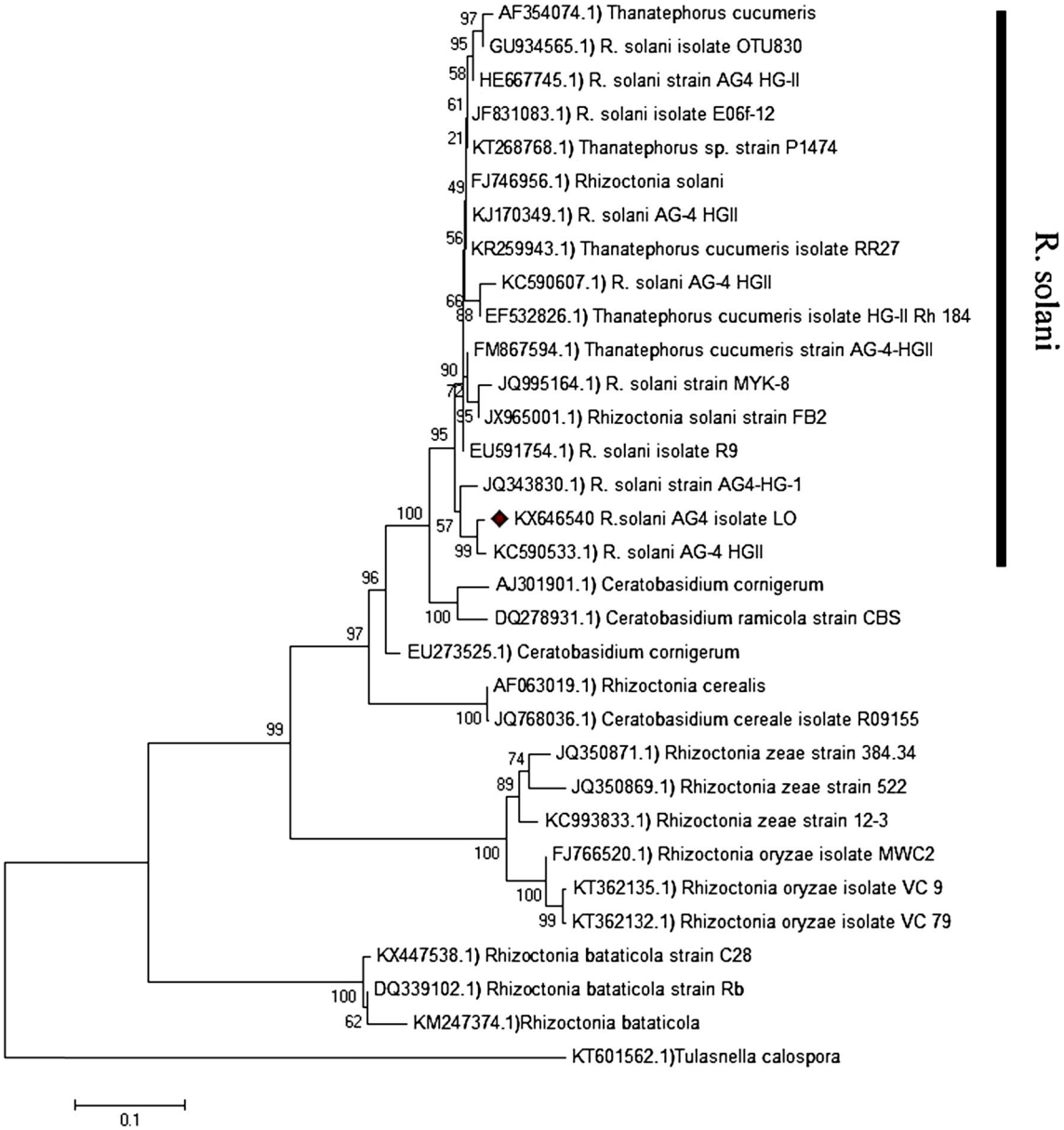

Fig. 3 Phylogenetic tree based on Neighbor-Joining analysis of ITS rDNA sequences of *Rhizoctonia solani* implemented by MEGA6 software. The isolate used in this study marked with red oval. *Tulasnella calospora* KT601562.1 was used to root the tree

Fig. 4 Pathogenicity test on barley, inoculated with autoclaved wheat grains colonised by *Rhizoctonia solani*. Foliar symptoms appeared after one month of growth

representative sequence from this study clustered with *R. solani* isolates and distinguished it from other *Rhizoctonia* species. Our isolate shared also a common clade with *R. solani* AG4 HGII (KC590533.1) isolated from wheat in Turkey (Fig. 3). Finally, Isolate LO was deposited in the Iranian Fungal Culture Collection, Iranian Research Institute of Plant Protection, Tehran, Iran (IRAN 2613 C2).

The pathogenicity test was conducted using wheat grains colonised with the fungus as the inoculum. For this, 200 g wheat grains were soaked in water, autoclaved and mixed with 5-mm-diameter mycelial plugs from 4-day-old PDA cultures. Inoculated wheat grains were mixed with soil (20% *W*/W) and surface sterilised (2% sodium hypochlorite) barley seeds were cultivated in five pots (10 seeds per pot) kept at 22 °C for a month. Non-inoculated autoclaved wheat grains were used as control. After one month, initial symptoms appeared on the leaf margins as chlorotic spots and symptoms progressed as brown necrotic stripe and scald like lesions through the leaves while no symptoms were observed on roots (Fig. 4). Controls showed no symptoms. The observations and results were completely with those in the fields. The fungus was reisolated from infected plants and all characteristics were similar to those of the original samples.

During our surveys, almost 40% of the barley fields were found to be affected by *R. solani* AG4. More sampling and research are needed to understand the disease status in Chaharmahal and Bakhtiari and other regions of Iran. To date, there is no record of barley foliar blight caused by *R. solani* AG4 in Iran and to the best of our knowledge this is the first report of this pathogen on barley fields in Iran.

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