Diseases Caused by Fungi and Fungus-Like Organisms

First Report of Brown Leaf Spot of Rice (*Oryza sativa*) Caused by *Bipolaris sorokiniana* in Pakistan

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Brown leaf spot of rice is one of the major seed-borne diseases and can diminish grain production by up to 52% (Barnwal et al. 2013). In 2018, infected leaf samples showing typical symptoms of brown spots were collected from the vicinity of the University of Agriculture, Faisalabad (31°26'10.3" N, 73°03'35.1" E). The symptoms were dark brown spots, with gray to light gray or brown centers surrounded by dark margins and with chlorotic halos of oval or cylindrical shapes (5 to 9 mm in diameter). Disease incidence averaged 61% across the seven fields observed. Leaves were collected from the seven infected fields, and symptomatic leaf tissues of 5 mm² were excised from representative necrotic spots in each leaf. These tissues were surface disinfected with 70% ethanol, rinsed with sterile distilled water (SDW), dried by blotting on paper, and placed on potato dextrose agar medium. For pathogen growth, the plates were placed at 25 ± 2°C with a 12-h photoperiod for 5 days. Five samples from each of the infected fields were taken for pathogen isolation, and 10 isolates among them were subcultured and purified by using the single-spore method. The resulting fungal colonies were fluffy and ranged in color from grayish black/black to light brown. Fifteen conidia were measured that were olivaceous/brown to dark brown in color, elliptical to oblong with narrow (tapered) ends, had 3 to 10 septa, and were 35.6 to 65.4 µm in length × 13.1 to 25.7 µm in width.

Conidiophores were yellowish to brown, geniculate, and solitary (Pratt 2003). For molecular studies, rDNA of the internal transcribed spacer (ITS) region, translation elongation factor (*tef*), RNA polymerase II second largest subunit (*rpb2*), and glyceraldehyde-3-phosphate dehydrogenase (*gpd*) gene were amplified by using the primers ITS1F/ITS4R (White et al. 1990), EF1-983F/EF1-2218R (Rehner and Buckley 2005), 5F2/7CR (O'Donnell et al. 2007), and GPD1/GPD2 (Berbee et al. 1999), respectively. The sequence of all the amplified gene regions of one SUL-1 isolate was deposited into GenBank with accession numbers MN314844 (ITS), MN326866 (*tef*), MN990457 (*rpb2*), and MN990456 (*gpd*). BLASTn queries of the obtained sequences (ITS, *tef*, *rpb2*, and *gpd*) showed 99 to 100% homology with the corresponding nucleotide sequences of *Bipolaris sorokiniana* (GenBank accession nos. GU480767, MF490855, LT715652, and MK558818, respectively). To fulfill Koch's postulates, 20 rice plants (cv. Basmati-385) were sprayed at two- to three-leaf stages by using the two representative isolates with a spore suspension of 10⁵ spores/ml. SDW was sprayed on 10 control plants. The plants were covered with polyethylene bags to keep the moisture contents and incubated at 25 ± 2°C for 7 days. After a week, the same symptoms as those described above were observed. In the repeated experiment, *B. sorokiniana* was reisolated from the infected rice leaves and confirmed morphologically, which fulfilled Koch's postulates. Worryingly, the other species of the genus *Bipolaris* (*B. oryzae*, and *B. victorae*) have also been found to cause brown leaf spot in rice (Motlagh and Kaviani 2008). To our knowledge, this is the first report of *B. sorokiniana* causing brown leaf spot of rice in Pakistan. Because rice is a highly consumable grain in Pakistan, the rapid spread of this disease in rice farming areas is a serious concern.

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