Diseases Caused by Fungi and Fungus-Like Organisms

First Report of Leaf Spot Disease on *Fagopyrum esculentum* Caused by *Bipolaris zeae* in China

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Common buckwheat (*Fagopyrum esculentum* Moench), a dicotyledonous plant in family Polygonaceae, is recognized as a valuable nutritional source of fatty acids, phytosterols, phenolic compounds, and tocopherols. It has received increased attention as a so-called functional food in China. During scouting of common buckwheat in August and September 2018, unfamiliar symptoms were observed on leaves in 20 fields in Yanchi County, Ningxia, China, with 35% incidence and moderate to high severity across the field. Brown spots most commonly occurred on lower leaves of buckwheat beginning in late July. The spots were initially light brown with an irregular border and pale brown center. Older spots were almost dark brown and often coalesced, although spots were restricted by veins. Symptomatic leaf samples were collected in late August and washed with flowing water for 2 min. Tissue samples were excised from the margins of the lesions and sterilized with 75% ethanol for 20 s and 0.1% NaClO for 2 min, before being rinsed with sterilized water four times, dried on sterile paper towels, and cultured on potato dextrose agar (PDA) medium at 20°C. After 48 to 72 h, hyphae grew from tissue pieces. The obtained isolates were purified using the single-spore isolation technique. A total of 13 isolates were recovered and characterized. Colonies of all isolates on PDA were identical and were pale brown to brown, velvety, with irregular borders. Hyphae were hyaline to brown, branched, septate, smooth, sometimes verruculose, 3 to 7 µm wide.

Conidiophores were 83 to 297 × 5.5 to 9 µm (average 205 × 8 µm, $n = 30$), dark brown, cylindrical, straight to slightly flexuous, geniculate at the upper end, septate, single, and smooth. Conidia were rostrate, obclavate, straight to slightly curved, hyaline to pale brown when immature and brown to reddish brown when mature, and measured 38 to 104 × 11 to 19 µm (average 67 × 16 µm, $n = 50$). End cells were often paler than middle cells and with a thick dark hilum. Based on the cultural and morphological characteristics, these isolates were preliminarily identified as *Bipolaris zeae* (Manamgoda et al. 2014; Sivanesan 1985). Genomic DNA of the representative isolate qyj-5A was extracted and amplified using GAPDH primers (gpd 1/2) and ITS primers (ITS 1/4) respectively. The sequence of the amplicons was compared with reference sequences. The ITS sequence (GenBank: MT645704) showed 100% (571/571 bp, 564/564 bp) identity with *B. zeae* (GenBank: KU356179 and KU571464, respectively). The GAPDH sequence (GenBank: MW426530) also showed 100% (594/594 bp) identity values with *B. zeae* (GenBank: MF415650 and KU571468). Pathogenicity tests were performed on three healthy and asymptomatic buckwheat plants, which were surface sterilized with 75% ethanol and rinsed with sterilized distilled water. The leaves were sprayed with 1×10^5 conidia/ml of the suspensions, which contained 0.01% Tween 20, and three control plants were sprayed with same volume of sterile distilled water. A strip of parafilm was wrapped around the inoculated leaves for 48 h to maintain high relative humidity. At 6 days post-inoculation, all the inoculated leaves showed symptoms identical to those described above, whereas no symptoms were observed on the control plants. The fungus was reisolated and identified as *B. zeae* based on morphological features and DNA sequence analysis, and it was identical to the original isolate to satisfy Koch's postulates. *B. zeae* has been reported to be pathogenic on *Acer truncatum* (Sun et al. 2011), *Helianthus tuberosus* (Zhao et al. 2017) and *Hemarthria altissima* (Xue et al. 2017) in China. To our knowledge, this is the first report of *B. zeae* causing leaf spot on *F. esculentum* in China. This fungal pathogen represents a severe threat and has the potential to cause yield losses of *F. esculentum*, so further research is required to define effective management strategies.

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