Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Grammothele lineata* Causing Stem Rot of *Areca catechu* in Hainan Province, China

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Areca catechu L. (areca) belongs to the Arecaceae family, which is composed of 181 genera and 2,600 species (Christenhusz and Byng 2016), and is cultivated extensively in southern and southeastern Asia (Peng et al. 2015). Areca has a long history for its important economic and medicinal benefits and is one of the most important commercial crops in Hainan Province, China. In recent years, root rot and stem rot diseases have occurred, causing areca plants to wither and even die. The serious symptoms mainly appeared in Hainan Province (Li et al. 2006). In March 2018, the rotten tissues of the diseased plants were observed becoming brittle, brown, and even black from the stem base to the root; the outer leaves turned yellow, dry, and dropped in areca plantations of Qionghai County. The disease can spread from individual plants to the whole plantation in one to two years, with the characteristics of large-scale occurrence and rapid transmission, causing huge economic losses. The diseased tissues (5 × 5 mm) were disinfected with 75% ethanol for 30 s, 1% HgCl₂ for 1 min, washed in sterile water, placed on potato dextrose agar (PDA) medium, and incubated at 28°C (Gao et al. 2019). Pure isolates were obtained by transferring the mycelium around the diseased tissues to PDA several times. The colonies were white and cottony after culturing for 7 days. The reverse side of the colony was yellowish white. Basidiospores were hyaline, thin-walled, smooth, 1.7 to 1.8 × 1.6 to 1.7 μm (*n* = 30) in size and circular or elliptical in shape, in addition to a dimitic hyphal system (Das et al. 2017). For molecular identification, the

genomic DNA of the isolate was extracted using the thermolysis method (Zhang et al. 2010). The ribosomal internal transcribed spacer (ITS) region was amplified using the primer pairs ITS1/ITS4 (White et al. 1990), and the amplified DNA fragments were sequenced. The obtained ITS (GenBank accession no. MW534416) had 99.36% identity with the reference sequence (GenBank accession no. KX013157) of *Grammothele lineata* Berk. & M.A. Curtis. A phylogenetic tree was constructed with MEGA7 software using the neighbor-joining method, showing that the isolate was grouped in the same clade as *G. lineata*. To fulfill Koch's postulates, a pathogenicity test was performed using the stems of 6-month-old healthy areca seedlings. Stem surfaces were sterilized with 70% ethanol for 30 s, rinsed three times with sterile water, gently stabbed with a sterile needle, and inoculated with 1-cm-diameter colonized PDA disks from a 7-day culture on wounds, moistened with wet cotton, and wrapped with fresh plastic wrap. Plants inoculated with sterile PDA plugs were used as the control. The inoculation assay was carried out twice, with five plants in both control and treatment in each test. After 20 days, the stems of the plants inoculated with the pathogen exhibited rotten symptoms, and the leaves began to become yellow and shrunken, while the control plants had only the surface of the stems discolored slightly and the inner tissue was undamaged. The fungus was reisolated from the infected stems. Based on the morphological observations and ITS sequence analysis, the isolate was identified as *G. lineata*. As far as we know, this is the first report of *G. lineata* causing stem rot of areca in China.

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