



NEW DISEASE REPORT

First report of *Diaporthe eres* causing leaf blight on vine tea in China

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Funding information

the Study on the Identification of Pests and Diseases and Green Control Technology on Vine Tea, Grant/Award Number: 5026401-0320012

KEYWORDS

diaporthe eres, leaf blight, vine tea

Vine tea or moyeam (*Nekemias grossedentata*) is a traditional herb widely consumed in China. A leaf blight with an incidence of up to 50% was observed in at least 10 vine tea plantations in Zhangjiajie, China in 2021, causing direct economic losses of more than US\$10 million in 2021. Initial symptoms on leaves were small, round, brown lesions. As the disease progressed, the lesions extended and merged with adjacent lesions, eventually leading to leaf blight. Black spots were observed on the leaves in the later stages of the disease (Figure 1).

125 leaf pieces with typical disease symptoms were sampled from eight different vine tea plantations in Zhangjiajie. Small pieces of tissue were cut from the margins of the lesions, disinfested in 3% NaClO for two minutes, rinsed with sterile water three times, and placed on water agar plates. After incubation at 28°C for five days, 90 isolates with similar morphological characteristics were obtained. All colonies were white with sparse, aerial mycelia, and became grey after five days' incubation on potato dextrose agar (PDA) plates (Figure 2). After



FIGURE 1 Typical symptoms of vine tea leaf blight observed in the field in Zhangjiajie, China. The pathogens caused small, round, brown lesions at the early stages of disease infection. As the disease progressed, the lesions extended and merged with adjacent lesions.

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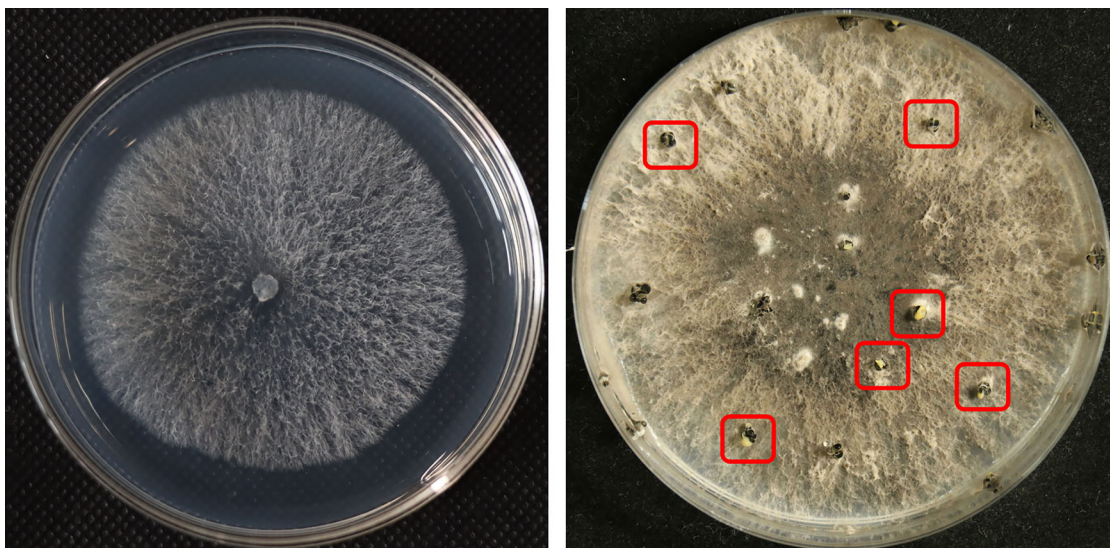


FIGURE 2 Colony morphology of *Diaporthe eres* (left) and formation of pycnidia (right).

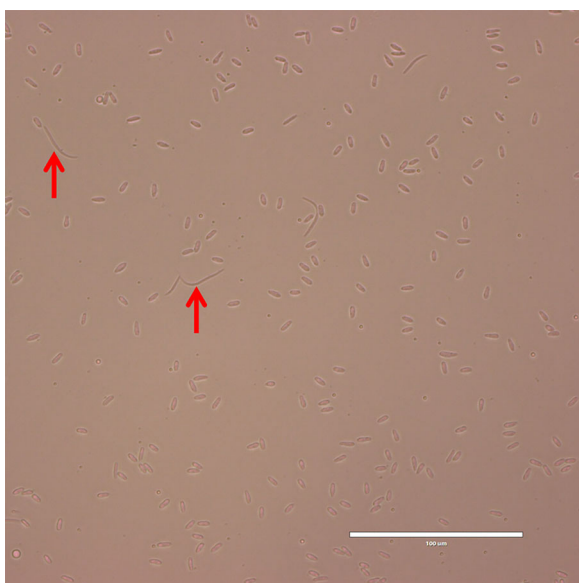


FIGURE 3 Conidia of *Diaporthe eres*. Bar = 100 μm .

30 days' incubation, black pycnidia formed on PDA plates, with yellowish, conidial cirrus extruding from the ostioles (Figure 2). Two types of conidia were observed. The α conidia were smooth, hyaline, fusoid, and mostly biguttulate, and measured 6.1 to 10.2×2.3 to 4.1 (mean 7.9×3.2) μm ($n = 60$). β conidia were smooth, aseptate, hyaline, fusiform to

hooked, and measured 19.3 to 28.6×1.0 to 1.4 (mean 24.9×1.2) μm ($n = 30$) (Figure 3).

Four isolates were selected randomly for molecular analysis to further identify the pathogen (A1, A3, A6 and A8). The partial *ITS*, *EF1- α* , *His* and *β -tubulin* genes were amplified with primers described previously (Glass & Donaldson, 1995; Udayanga et al., 2014). Phylogenetic trees were constructed using concatenated *ITS*, *TUB*, *HIS* and *EF1- α* sequences based on the neighbour-joining method. All four isolates clustered with *Diaporthe eres* isolate SH-CW1 but separated into different clades (Figure 4), suggesting that the fungi are genetically different.

Koch's postulates were carried out in greenhouse experiments with the four *D. eres* isolates. Five-day-old mycelial plugs of *D. eres* isolates were inoculated on needle-wounded leaves of vine tea plants, using 10 replicates for each fungal isolate. All plants were placed in an incubator under 25°C and 80% humidity. Five days after inoculation, the leaves inoculated with *D. eres* exhibited dark-brown spots (Figure 5). No disease symptoms developed in non-inoculated plants. All the isolates recovered from the inoculated plants exhibited similar morphological characteristics to the inoculum.

Although *D. eres* has been reported to cause leaf blight/spot on different crops (Song et al., 2018; Zheng et al., 2019; Zhang et al., 2022), to our knowledge, this is the first report of *D. eres* causing leaf blight on vine tea in China.

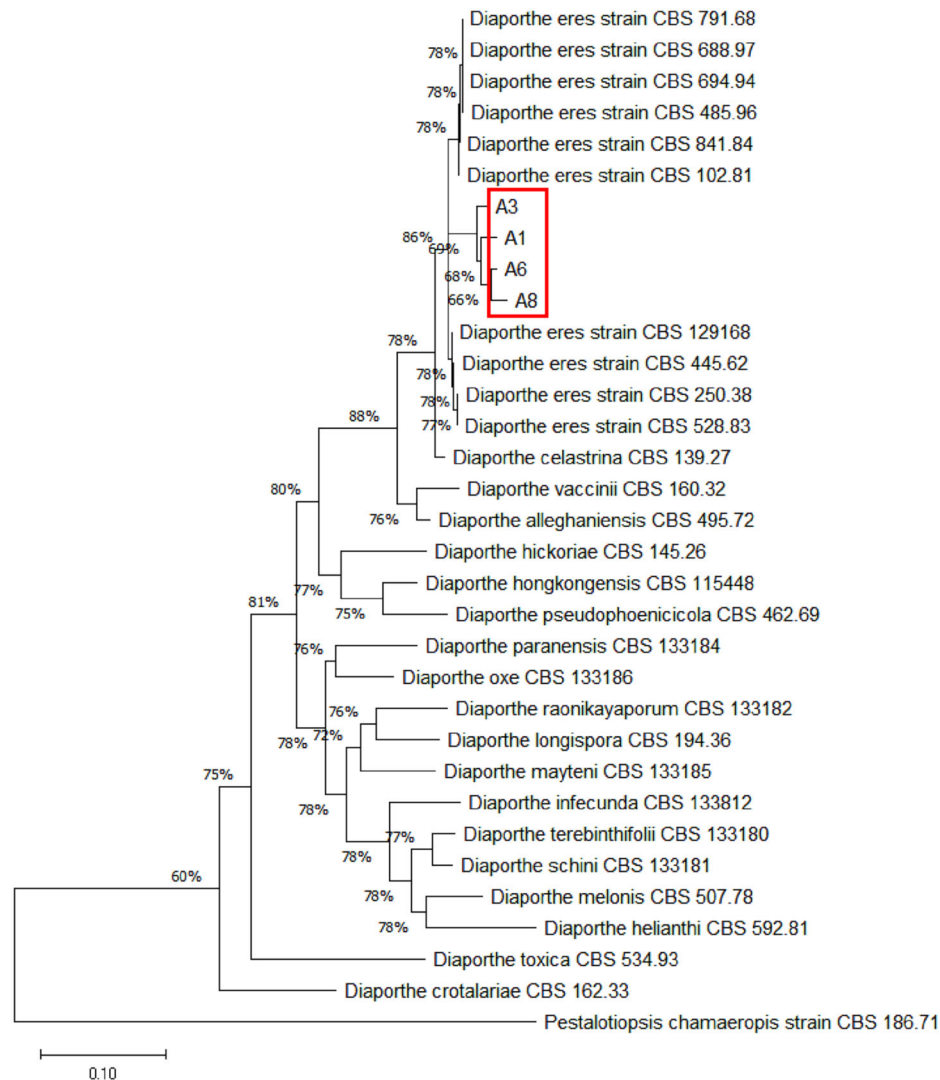


FIGURE 4 Phylogenetic tree based on the concatenated sequences of *ITS*, *TUB*, *HIS* and *EF1-α* genes of the isolates A1 (GenBank Accession Nos. OP326159, OP351550, OQ325322 and OQ325317), A3 (OP326160, OP351551, OQ325323 and OQ325318), A6 (OP326162, OP351553, OQ325325 and OQ325320), A8 (OP326163, OP351554, OQ325326 and OQ325321) from this study, 13 *Diaporthe* isolates and one *Phomopsis* isolate. *Neopestalotiopsis clavispora* was used as an outgroup.



FIGURE 5 Pathogenicity test of *Diaporthe eres* isolates (A1, A3, A6 and A8) showing disease symptoms five days after inoculation on wounded leaves.

ACKNOWLEDGEMENTS

This work was supported by the Study on the Identification of Pests and Diseases and Green Control Technology on Vine Tea of Zhangjiajie (grant number 5026401-0320012).

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How to cite this article: Liu, Y.J., Zhou, Z.H., Wang, Y.C., Liu, S., Deng, W.C., Hu, W.J. et al. (2024) First report of *Diaporthe eres* causing leaf blight on vine tea in China. *New Disease Reports*, 49, e12275. <https://doi.org/10.1002/ndr2.12275>