Received: 9 June 2023 | Accepted: 9 June 2023

DOI: 10.1002/ndr2.12192

New Disease Reports Open Access BSPP WILEY

NEW DISEASE REPORT

First report of Fusarium madaense as a cause of root and stalk rot on Sorghum bicolor in Australia

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Funding information
Broadacre Cropping Initiative in Queensland, Australia, Grant/Award Number: BACI52

KEYWORDS
Fusarium fujikuroi species complex, phylogenetic analysis

Sorghum (*Sorghum bicolor*) is mainly grown for stock feed in eastern Australia where significant economic losses result from diseases caused by *Fusarium* spp. (Petrovic et al., 2009; Kelly et al., 2017). During the 2018 sorghum growing season, 66 plants showing symptoms typical of *Fusarium* infection were randomly collected from three sorghum fields in Barramornie (27.08°N, 150.11°E), southern Queensland. *Fusarium* isolates were obtained from surface-sterilised sorghum stem or root pieces (1 cm) placed on *Fusarium*-selective PCNB media (Leslie & Summerell, 2008) and incubated at 23°C for five days. Ninety single-spore cultures were sub-cultured on potato dextrose agar (PDA) for molecular characterisation and 17 of these were deposited into the Queensland Plant Pathology Herbarium (BRIP, Brisbane, Australia).

One isolate (BRIP 75161a) recovered from a diseased stem node was identified as *F. madaense* by DNA barcodes. Comparison of the partial gene sequences of RNA polymerase II largest subunit (*rbp1*), RNA polymerase II second largest subunit (*rbp2*), and translation elongation factor 1-alpha (*tef-1α*) (GenBank Accession Nos. OQ695493, OQ657300 and OQ657299, respectively) of isolate BRIP 75161a showed 100%, 100% and 99.7% nucleotide identities with *F. madaense* (ex-type: CBS 146669), respectively. The polyphasic identification tool on the Mycobank FUSARIOID-ID database (https://www.mycobank.org) using the same three genes confirmed the identity of the isolate as *F. madaense* with 99.9% identity to *F. madaense* (ex-type: CBS 146669). Phylogenetic analysis with partial sequences of *rbp1*, *rbp2* and *tef-1α*, with five closely related species (*F. andiyazi*, *F. madaense*, *F. mirum*, *F. musae*, and *F. verticillioides*) (Costa et al., 2021a) resulted in isolate BRIP 75161a clustering with the ex-type strain and reference isolates of *F. madaense* in a well-supported clade (Figure 1).

Colonies of *F. madaense* (BRIP 75161a) on synthetic nutrient-poor agar and carnation leaf agar (Leslie & Summerell, 2008) were white with infrequent areal mycelium without pigmentation (Figure 2), while on PDA they were powdery to floccose with abundant whitish aerial mycelia, becoming violet. Microconidia were cylindrical to narrowly ellipsoidal or obovoid, 0–4 septate, 6–16 × (1.5–) 2–4.5 μm, hyaline, produced on false heads (Figure 3) or long aerial chains.

Pathogenicity was assessed on 10-day-old seedlings of *S. bicolor* cv. Resolute. One plant per pot was grown in sterile vermiculite

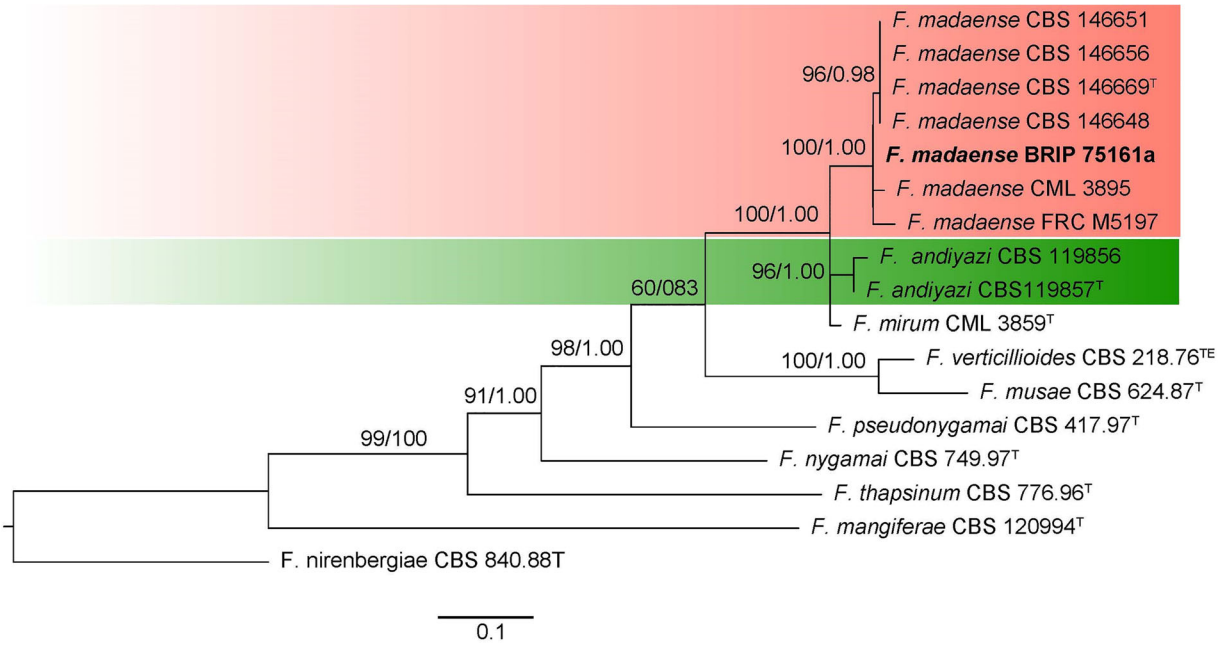

FIGURE 1 Maximum likelihood phylogenetic tree inferred from a concatenated alignment including partial sequences of *tef-1α*, *rbp2*, and *rbp1*, with *Fusarium nirenbergiae* (ex-type strain CBS 840.88) as the outgroup depicting the phylogenetic relatedness of *Fusarium madaense* with other species within the *Fusarium fujikuroi* species complex. Trees were generated treating each gene region as separate partitions using Maximum Likelihood (ML) (RAxML v 8, GTR-GAMMA evolution model with 1000 bootstrap replicates), and Bayesian Inference (BI) analyses (MrBayes v 3.2.6 after selecting the best-fit nucleotide models using Akaike information criterion in MrModeltest v 2.3. [SYM+G for *tef-1α* and *rbp1*, and GTR+I for *rbp2*]). ML bootstrap and Bayesian posterior probability values are shown at the internodes. Accession number in bold font refers to the isolate from this study. Sequences from ex-type and ex-epitype strains are indicated with ^{T} and^{ET}, respectively.

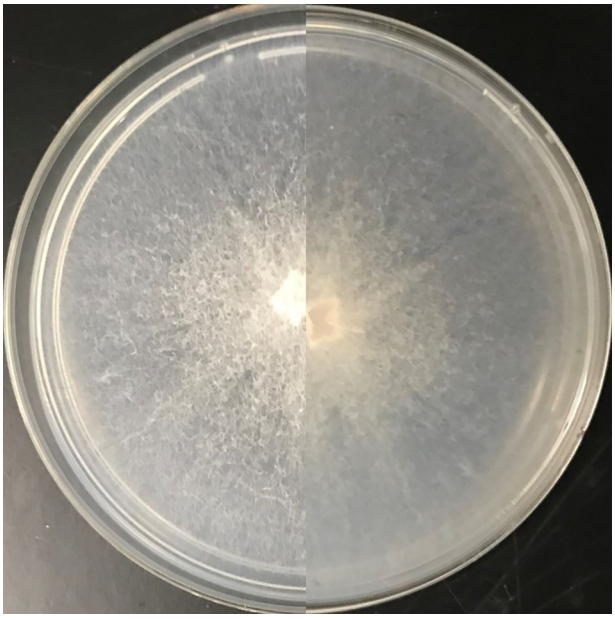

FIGURE 2 Morphology of *Fusarium madaense*. A 10-day-old colony on synthetic nutrient-poor agar: upper surface (left) and lower surface (right)

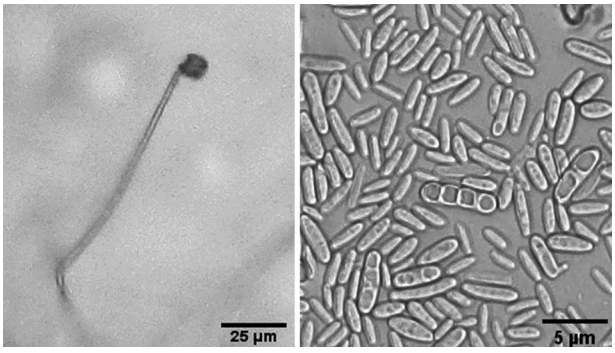

FIGURE 3 Aerial conidiophore of *Fusarium madaense* with (a) false head and (b) microconidia produced on carnation leaf agar

inoculated with an agar plug (0.5 cm) of isolate BRIP 75161a (Kelly et al., 2017). Each experiment consisted of three replicates and the same experiment was conducted three times. Reddish to black discolouration in infected roots were observed (Figure 4). Control plants grown in sterile vermiculite inoculated with a non-colonised agar plug did not develop symptoms (Figure 4). Koch's postulates were completed by reisolating the fungus from the root tissues (10 days post inoculation) and by comparing cultural morphology on PDA.

Fusarium madaense causes stalk rot in sorghum, maize, and millet, and pokkah boeng in sugarcane (Costa et al., 2021a). It was first isolated from groundnut and sorghum in Nigeria (Ezekiel et al., 2020) and later identified in several tropical countries (Costa et al., 2021b). This is the first report of *F. madaense* in Australia.

FIGURE 4 Root symptoms caused by *Fusarium madaense* (BRIP 75161a) on 10-day-old seedlings of *Sorghum bicolor* cv. Resolute: (a) dark red to black discolouration of diseased roots, and (b) healthy root system

ACKNOWLEDGEMENTS

This work was carried out as part of a project funded by Broadacre Cropping Initiative in Queensland, Australia.

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How to cite this article: Gunasinghe, N., Vaghefi, N., Shivas, R.G., Tan, Y.P., Jordan, D. & Mace, E. et al. (2023) First report of Fusarium madaense as a cause of root and stalk rot on Sorghum bicolor in Australia. *New Disease Reports*, 47, e12192. https://doi.org/10.1002/ndr2.12192