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NEW DISEASE REPORT

Clonostachys chloroleuca: A novel pathogen causing cassava root rot disease in Misiones Province, Argentina

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Cassava (*Manihot esculenta*) is a vital food crop but its production is threatened by Cassava root rot disease (CRRD) which is caused by a range of fungi including *Fusarium*, *Lasiodiplodia* and *Phytophthora* (Teixeira et al., 2021). In July 2020, storage roots from three cassava plants exhibiting symptoms of CRRD (Figure 1) were collected for microbial diagnosis from a cultivated field situated in the southwest of the subtropical Misiones Province, Argentina (27°30' S; 55°45' W). The plants exhibited aerial symptoms, including reduced plant height, chlorosis and wilting of the leaves, and poor storage root development. The fungal isolation was done following the protocol of Sangpueak et al. (2023). Morphological identification involved cultivating the fungus on potato dextrose agar (PDA) at 28±2°C. Macromorphological and microscopic characteristics, including primary and secondary conidiophores as well as conidia, were examined and matched descriptions of *Clonostachys chloroleuca* (Moreira et al., 2016) (Figure 2). For molecular identification, the Internal Transcribed Spacer (ITS) and the translation elongation factor 1 alpha (*tef 1-α*) loci were sequenced (GenBank Accession Nos. OR373883 and OR402898, respectively) and phylogenetic analysis using the Maximum Likelihood method with Jukes Cantor model confirmed the isolated fungus (isolate MEL2) as *Clonostachys chloroleuca* (Figure 3).

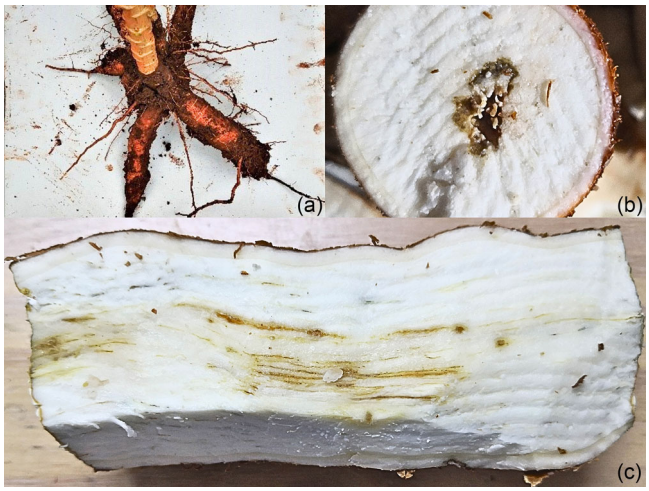

FIGURE 1 Cassava root rot disease on cassava collected in Misiones Province, Argentina: (a) poorly developed cassava roots with characteristic dry root; (b) root cross-sectional view, showing dry consistency of the pulp with tissue degradation and blackening in the centre; and (c) root longitudinal view, with black streaking extending along the length of the root.

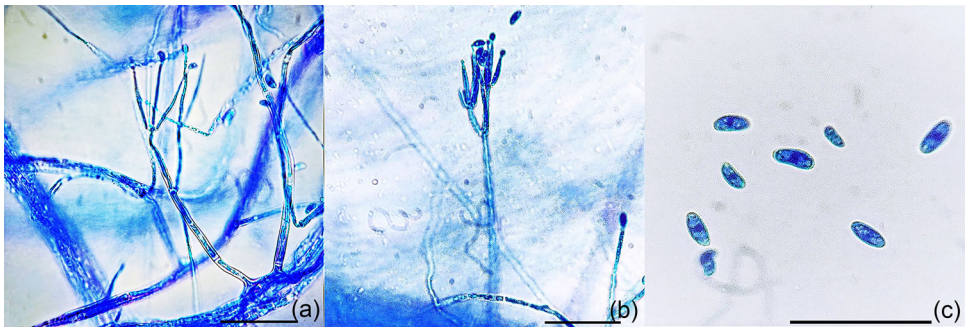

FIGURE 2 Microscopic characteristic of *Clonostachys chloroleuca* isolate MEL2: (a) primary conidiophores; (b) secondary conidiophores; and (c) conidia. Bar = 25 μm.

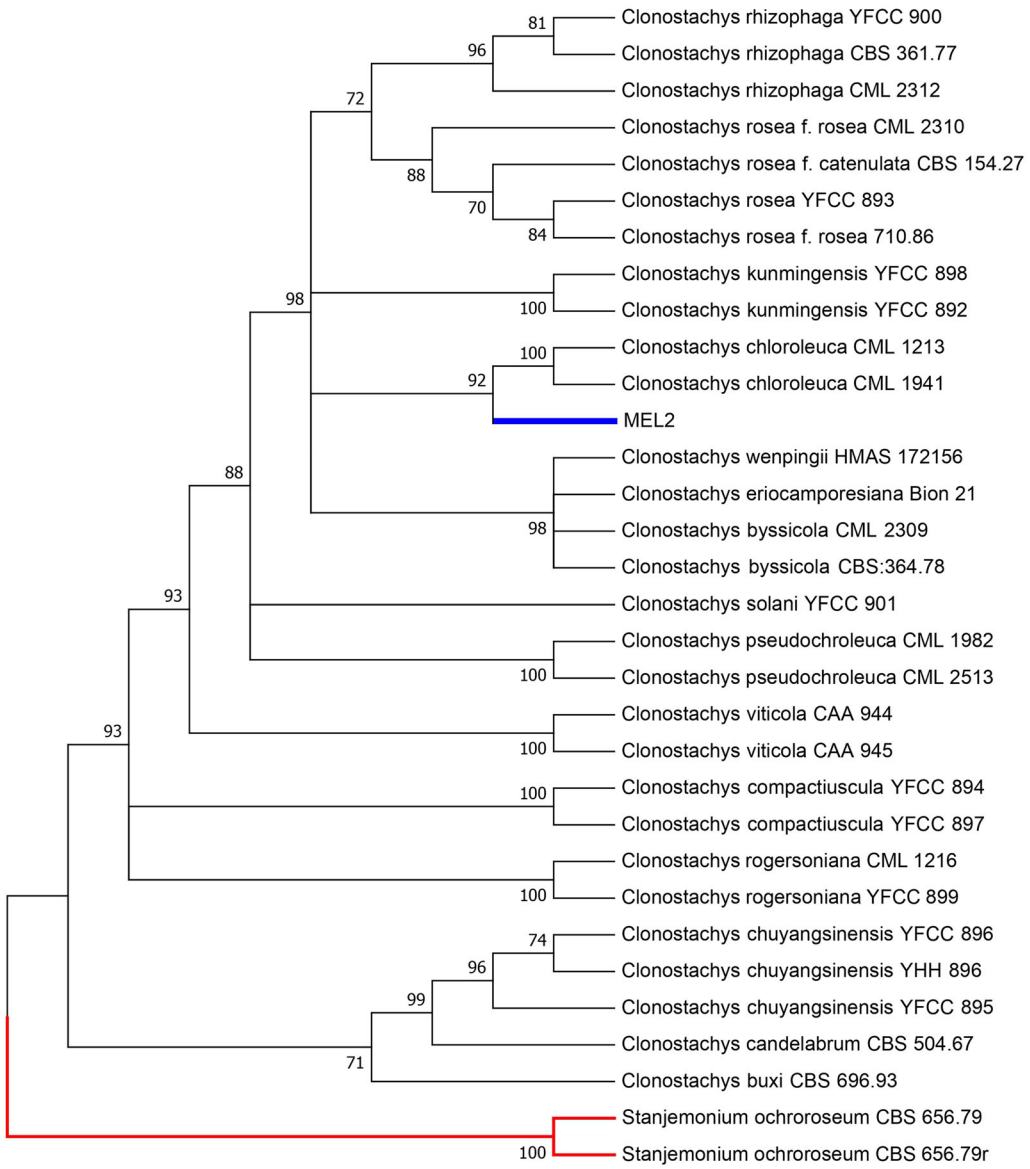

FIGURE 3 Maximum Likelihood tree for combined sequences of the ITS and the *tef 1-α* regions for *Clonostachys* species. Accession number CML 1941 represents the type strain of *C. chloroleuca*; blue line indicates isolate MEL2. Sequences from *Stranjemonium ochroroseum* were used as outgroups (red lines). Only branches with bootstrap values >70 are shown.

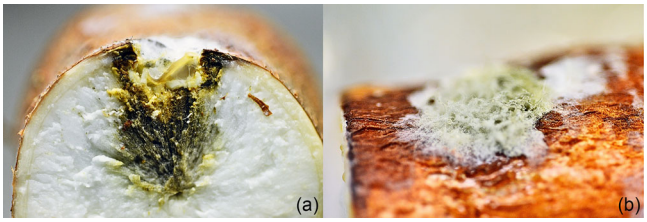

FIGURE 4 Cassava root rot symptoms caused by *Clonostachys chloroleuca* isolate MEL2 In vitro: (a) root cross-sectional view, dry consistency and blackening of the pulp; and (b) Superficial, greenish mycelia.

In vitro pathogenicity tests, done according to Brito et al. (2020) with five repetitions, revealed that 22% of the cross-sectional area of storage roots infected with isolate MEL2 was symptomatic. The rot tissue was black and the pulp had a dry consistency (Figure 4a). The fungus also grew vigorously on the surface of the roots (Figure 4b). To assess pathogenicity in vivo, assays were conducted under greenhouse conditions (25±2°C, with 80% relative humidity and a 12:12 hour light-dark cycle) using cassava plantlets (produced from surface-sterilised cuttings) in 5 litre pots containing sterilised soil. At the time of planting, the cassava cuttings were treated with 10 mL of a conidial suspension (10^6 conidia/ml) prepared from two-week-old fungal colonies of isolate MEL2 grown on PDA. Inoculated 90-day-old cassava plants exhibited typical aerial CRRD symptoms while non-inoculated control plants remained asymptomatic. Microbial isolation performed on the roots inoculated in vitro and in vivo and subsequent morphological and molecular analysis of resulting fungal colonies (as outlined above) confirmed the presence of *C. chloroleuca* in inoculated plants, but not in non-inoculated ones, fulfilling Koch's postulates.

This finding highlights the importance of considering *C. chloroleuca* as a potential threat to cassava production and the necessity of further research on its epidemiology and management strategies. *Clonostachys chloroleuca* was first described in Brazil by Moreira et al. (2016) and reported as a plant pathogen in chickpea (*Cicer arietinum*) by Cota-Barreras et al. (2022). This is the first report of *C. chloroleuca* as a putative causative agent of CRRD. The symptoms induced by this pathogen differ from those previously described for CRRD, especially regarding the black colouration in the rotten pulp. Further studies are required to understand the pathogenic mechanisms of *C. chloroleuca* isolate MEL2 in causing CRRD, and field experiments are needed to validate the findings in real-world conditions.

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