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NEW DISEASE REPORT

# Molecular identification and pathogenicity of *Curvularia spicifera* isolated from seeds of the hybrid elephant grass cultivar BRS Capileto (*Pennisetum purpureum* × *Pennisetum glaucum*) in Brazil

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Elephant grass is a major forage crop, widely used to feed dairy herds in Brazil. In April 2023, 200 seeds of cv. BRS Capileto (*Pennisetum purpureum* × *Pennisetum glaucum*), a commercial elephant grass hybrid developed by Embrapa, were collected from a seed crop in the municipality of Coronel Pacheco, Minas Gerais, Brazil. The seeds were collected at harvest for a scheduled phytosanitary evaluation. Seeds were divided into four groups of fifty. Each group was disinfected by submerging in a 1% NaClO solution, for three minutes and then, subsequently, washed in sterile water. Each group was distributed between ten Petri dishes, five seeds per plate, containing potato dextrose agar + streptomycin medium. The plates were incubated in a growth chamber

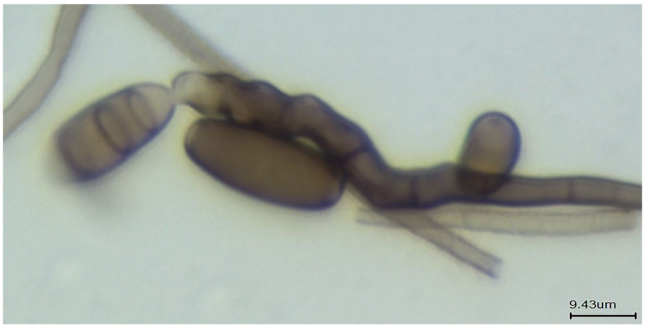


**FIGURE 1** Conidiophore of *Curvularia spicifera* isolated from hybrid elephant grass cv. BRS Capileto.

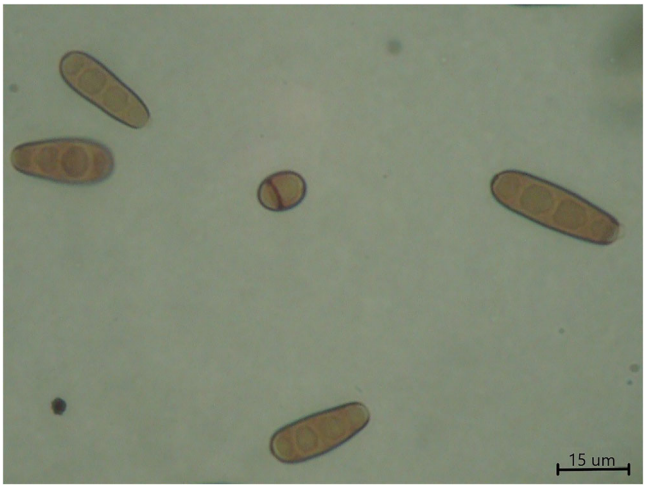


**FIGURE 2** Conidia of *Curvularia spicifera* isolated from hybrid elephant grass cv. BRS Capileto.

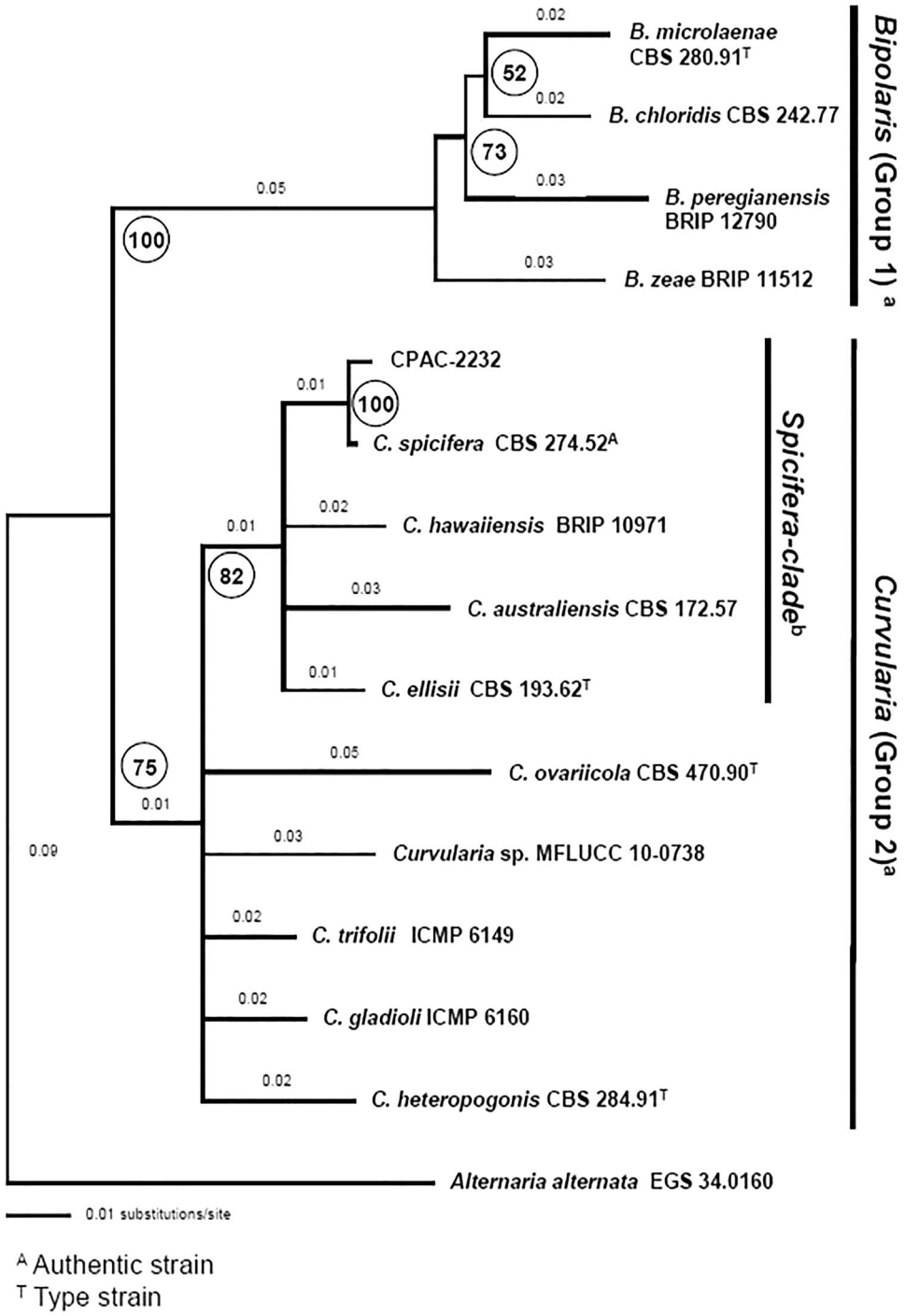


**FIGURE 3** Neighbour-joining phylogenetic tree based on the combined dataset of ITS and GPDH gene sequences. The tree was rooted to *Alternaria alternata*. Maximum likelihood bootstrap support values (>50%) from 1,000 replicates are indicated at the nodes. Accession numbers of ITS and GPDH sequences of the strains used in this analysis can be found in Berbee *et al.* (1999), Manamgoda *et al.* (2011), Manamgoda *et al.* (2012) and Tan *et al.* (2014). [a]Classification by Manamgoda *et al.* (2012). [b]Classification by Madri *et al.* (2014).

at 30 +/-2°C with a 12 hour photoperiod for seven days. Seed germination was 30% and the same fungus grew from the seeds in all plates. The fungus presented conidiophores, singly or in groups, brown to dark brown in colour, flexible, with septa and scars (Fig. 1). The conidia, produced at the apex of the conidiophores, were straight and brown with rounded ends, cylindrical with three pseudosepta (Fig. 2). The dimensions ranged from 20.0-28.8 × 7.5 to 12.5 µm ($n$ = 100, mean = 25.0 × 10.6 µm). These characteristics match the description of *Curvularia spicifera* (Ellis, 1971). The fungus was isolated in pure culture and one isolate (CPAC-2232) was used for further study.

The DNA of isolate CPAC-2232 was extracted using the CTAB method for fungi (Zolan & Pukkila, 1986). The glyceraldehyde-3-

**FIGURE 4** Leaf lesions caused by *Curvularia spicifera* in hybrid elephant grass cv. BRS Capileto elephant grass, seven days after artificial inoculation.

phosphate dehydrogenase (GPDH) and internal transcribed spacer (ITS) sequences were amplified by PCR using the gpd1/gpd2 (GenBank Accession Number: MT497471) and its4/its5 (PP707943) primer pairs, respectively (Berbee *et al*., 1999), and analysed in PAUP v4.0b10 (Swofford, 2002) using the neighbour joining method. Phylogenetic analysis of the concatenated ITS and GPDH sequences from CPAC-2232 with a thousand bootstrap repetitions confirmed the identification as *C. spicifera* (Fig. 3). A BLASTn search showed individual ITS and GPDH sequences have 100% and 99.41% identity, respectively, to homologue sequences of *C. spicifera* isolate CBS 274.52 (JN192387 and JN600979).

Monosporic cultures of isolate CPAC-2232 were incubated in V8 medium at 30 +/-2°C with a 12-hour photoperiod for seven days, for use in pathogenicity tests. The fungal spores were washed with sterile distilled water forming a spore suspension, adjusted to a concentration of $1.0\times10^6$/ml conidia, with a total volume of 400 mL. The suspension was sprayed with a manual atomiser on twenty grass seedlings of cv. BRS Capileto at the 2–4 leaf-stage, in one-litre pots. An equal number of healthy plants were sprayed with sterile water as controls. The pots were kept in trays containing water, inside an incubation chamber at 28 +/-2°C, for 48 hours, and then transferred to a greenhouse. The experiment was repeated twice. Four days after inoculation, all plants showed brown, irregularly-shaped lesions, which coalesced as they increased in quantity and size (Fig. 4). *Curvularia spicifera* was reisolated from these lesions three days later and identified using the molecular methods described previously.

*Curvularia spicifera* has been associated with a wide variety of hosts, mainly in tropical and subtropical countries (Ellis, 1971). To the best of our knowledge, this is the first report of this pathogenic fungal species associated with forage grass seeds in Brazil.

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## REFERENCES

Berbee, M., Pirseyedi, M. & Hubbard, S. (1999) *Cochliobolus* phylogenetics and the origin of known, highly virulent pathogens, inferred from ITS and glyceraldehyde-3-phosphate dehydrogenase gene sequences. *Mycologia*, 91, 964–977. https://doi.org/10.1080/00275514.1999.12061106

Ellis, M.B. (1971) *Dematiaceous Hyphomycetes*. Kew, UK: Commonwealth Mycological Institute. https://doi.org/10.1079/9780851986180.0000

Madrid, H., da Cunha, K.C., Gené, J., Dijksterhuis, J., Cano, J., Sutton, D.A. *et al*. (2014) Novel *Curvularia* species from clinical specimens. *Persoonia*, 33, 48–60. https://doi.org/10.3767/003158514x683538

Manamgoda, D.S., Cai, L., Bahkali, A.H., Chukeatirote, E. & Hyde, K.D. (2011) *Cochliobolus*: an overview and current status of species. *Fungal Diversity*, 51, 3–42. https://doi.org/10.1007/s13225-011-0139-4

Manamgoda, D.S., Cai, L., McKenzie, E.H.C., Crous, P.W., Madrid, H., Chukeatirote, E. *et al*. (2012) A phylogenetic and taxonomic re-evaluation of the *Bipolaris* - *Cochliobolus* - *Curvularia* complex. *Fungal Diversity*, 56, 131–144. https://doi.org/10.1007/s13225-012-0189-2

Swofford, D. (2002) *PAUP 4.0 b10: phylogenetic analysis using parsimony*. Sunderland, USA: Sinauer Associates.

Tan, Y.P., Madrid, H., Crous, P.W. & Shivas, R. G. (2014) *Johnalcornia* gen. et. comb. nov., and nine new combinations in *Curvularia* based on molecular phylogenetic analysis. *Australasian Plant Pathology*, 43, 589–603. https://doi.org/10.1007/s13313-014-0315-6

Zolan, M.E. & Pukkila, P.J. (1986) Inheritance of DNA methylation in *Coprinus cinereus*. *Molecular and Cellular Biology*, 6, 195–200. https://doi.org/10.1128/mcb.6.1.195-200.1986

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