

## Diseases Caused by Fungi and Fungus-Like Organisms

### First Report of Rice Brown Spot Caused by *Exserohilum rostratum* in Mali

Kouka Hilaire Kaboré,<sup>1,2,3</sup> Diariatou Diagne,<sup>2,3,4</sup> Joëlle Milazzo,<sup>2,3</sup> Henri Adreit,<sup>2,3</sup> Marc-Henri Lebrun,<sup>1</sup> and Didier Tharreau<sup>2,3,†</sup>

<sup>1</sup> UMR BIOGER, Université Paris-Saclay, INRAE, AgroParisTech, 78850 Thiverval-Grignon, France

<sup>2</sup> CIRAD, PHIM, 34398 Montpellier, France

<sup>3</sup> PHIM, Université Montpellier, CIRAD, INRAE, IRD, Montpellier SupAgro, Montpellier, France

<sup>4</sup> Laboratoire de Biologie Moléculaire Appliquée (LBMA)/Université des Sciences des Techniques et des Technologies de Bamako (USTTB), Mali

**Funding:** Support was provided by RICE CRP (grant to CIRAD), the Centre de Coopération Internationale en Recherche Agronomique pour le Développement (grant to K. H. Kaboré) and the Islamic Bank of Development (Ph.D. grant to K. H. Kaboré). Plant Dis. 106:1747, 2022; published online as <https://doi.org/10.1094/PDIS-03-21-0662-PDN>. Accepted for publication 11 November 2021.

Rice brown spot is an emerging disease of concern in many rice-growing countries. Different fungal species of the genera *Bipolaris* and *Exserohilum* were reported as the causal agents of this disease. These fungal pathogens cause similar necrotic lesions on leaves and infect grains with a significant effect on seed germination. In 2018, samples of rice seed and leaves with typical brown spot symptoms were collected from irrigated (Manikoura and Niono) and lowland (M'pegnesso and Loulouni) rice fields in Mali and incubated for 5 to 7 days on wet filter paper at 25°C with 12 h photoperiod. Conidia observed under a microscope were straight or slightly curved and light-brown or dark. They were also rostrate or obclavate and measured 31.4 to 275.6 × 7.3 to 18 µm ( $n = 40$ ). These morphological characteristics are identical to those of *Exserohilum rostratum* (Hernández-Restrepo et al. 2018). DNA from eight single-spored isolates was extracted by a CTAB-based protocol (Doyle and Doyle 1987). Internal transcribed spacer (ITS) rDNA region, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), and translation elongation factor 1 alpha (TEF1- $\alpha$ ) genes were amplified by PCR with the primers ITS5/ITS4 (White et al. 1990), GPD1/GPD2 (Berbee et al. 1999), and EF1 983/EF1 2218 (Rehner et al. 2005), respectively. The amplicons were sequenced and deposited in NCBI GenBank. Sequence similarity between the Malian strains

was 100% for ITS and GAPDH, and 99.8 to 100% for TEF1. Similarity between sequences of Malian strains and *E. rostratum* reference strains BRIP 11417 (GenBank acc. no. LT837836, LT882553, and LT896656) and CBS 128061 (GenBank acc. no. KT265240, LT715900, and LT896658) were 99.6 to 100%. A maximum-likelihood phylogenetic tree generated with ITS, GAPDH, and TEF1- $\alpha$  concatenated sequences using MEGA-X 10.1.7 grouped all eight strains from Mali in the *E. rostratum* clade with a bootstrap value of 100%. For a pathogenicity test, four strains from leaves and seed were grown on rabbit food agar (50 g/liter steeped filtrate of rabbit food pellets, Kaytee Products, Chilton, WI, USA, and 15 g agar) for 14 days at 25°C with a 12 h photoperiod (Hau and Rush 1980). Spores were collected and the concentration of spore suspension adjusted to  $1.5 \times 10^5$  conidia/ml with 0.5% gelatin. The rice varieties ADNY 11, ARICA 9, and Shwetaskoké were grown in pots with peat soil and NPK 13-5-18 at 3.5 g/liter of soil for 21 days. Four pots of each variety (5 seedlings/pot) were placed in a tray (60 plants per tray) and the leaves were sprayed with 30 ml of the conidial suspension or water at 0.5% gelatin (negative control). Plants were kept at maximum humidity (100%) at 21°C for one night and then transferred to a phytotron at 27°C. Seven days after inoculation, circular or oval foliar lesions less than 5 mm long, either brown or dark, sometimes whitish in their centers were observed. These lesions were identical to those observed in the field. *E. rostratum* was reisolated from these lesions. *E. rostratum* affects a wide range of plant species, particularly grasses, and has been observed on rice in many countries (Cardona and González 2007; Mahmad Toher et al. 2016; Majeed et al. 2016; Silva et al. 2016). However, to our knowledge, this is the first report of *E. rostratum* causing brown spot in rice in Mali.

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The author(s) declare no conflict of interest.

**Keywords:** cereals and grains, field crops, fungi, pathogen detection

<sup>†</sup>Indicates the corresponding author.  
D. Tharreau; tharreau@cirad.fr