Australasian Plant Disease Notes (2023) 18:30
https://doi.org/10.1007/s13314-023-00516-z

First report of *Curvularia akaiiensis* as a causal agent of leaf spot disease on Vetiver

Marlina Puspita Sari[1] · Dono Wahyuno[1] · Siti Hardiyanti[1] · Miftakhurohmah[1]

Received: 23 June 2023 / Accepted: 3 September 2023 / Published online: 8 September 2023

Abstract
Vetiver is currently cultivated in Indonesia; however, it is susceptible to leaf spot disease. Although usually minor, this hampers growth under favourable conditions. Through morphology and genetics study, we identified *Curvularia akaiiensis* as the causative agent, marking the first documentation of its role in vetiver leaf spot disease.

Keywords Fungal detection · Anamorph · Poaceae

Vetiver (*Vetiveria zizanoides*) is a perennial grass that has been widely recognised for its ability to address various environmental issues, including slope stabilisation, waste management, and phytoremediation of polluted land (Banerjee 2016). Within the first year of planting, the roots of the plant can grow rapidly, reaching depths of up to 3 to 4 m. The plant can produce vetiver oil, specifically Java vetiver oil. Indonesia is one of the three largest producers of vetiver oil worldwide, alongside Haiti and Pacific Region (Grover et al. 2021).

In the field, the vetiver plant is frequently affected by a leaf spot disease. While a relatively minor ailment for vetiver, under favorable environmental conditions and in high disease intensity, it can impede the plant's growth. The identification and characterisation of the pathogen responsible for the disease have not been previously reported in Indonesia (Semangun 1992; Kobayashi 1993). The symptoms primarily manifest on young leaves (Fig. 1A), which exhibit brown-black spots that extend along the leaf veins. These spots begin at the leaf edges and gradually spread towards the midrib.

Vetiver leaves with leaf spot symptoms were collected from Bogor, Indonesia. They were incubated at room temperature in moist conditions under light for 3 days to induce sporulation. The conidia that appeared were collected using a needle and dipped in sterile water in a test tube (18 × 180 mm). The conidial suspension was used for isolation and observation under a microscope. A single spore was isolated by scraping a sterile needle that had been dipped in a suspension of conidia on an aqueous agar medium and then incubated for 12 h (25°C). After 12 h, the germinated conidia were transferred to PDA medium (Chomnunti et al. 2014).

The isolation of a single spore on PDA medium yielded greyish mycelium colonies (Fig. 1B C). The morphological characteristics of the fungus were observed following the methodology outlined by Manamgoda et al. (2011) along with the approach described by Sivanesan in 1987, utilising a light microscope equipped with a digital camera. Sporulation began after 20 days of incubation under light at room temperature. The conidia had straight to slightly curved shapes and three septa (divisions), same as the conidia observed during the initial isolation. The central cell of the conidia was larger compared to the other cells. The sizes of the conidia ranged from 25 to 27 µm in length and 8 to 15 µm in width. The hilum of the basal cells was distinctly visible (protuberant). The conidial cell wall had a brownish colour, and the two end cells of the conidia were lighter in colour compared to the other cells (Fig. 1E). The conidiophores were cylindrical, predominantly unbranched, and possessed partitions (Fig. 1D). Optimal growth of the fungus colony occurred at a temperature range of 25–28 °C, with sluggish growth both above and below this temperature range. Based on the characteristics of the conidia and the conidiophores, this fungus belongs to the *Curvularia*

✉ Marlina Puspita Sari
marlina_puspita434@yahoo.co.id

[1] Research Center for Horticultural and Estate Crops, National Research and Innovation Agency, Cibinong Science Center, Jln. Raya Jakarta-Bogor, Bogor, West Java, Indonesia

Fig. 1 Vetiver leaves exhibiting leaf spot symptoms **(A)**, Conidia and Conidiofor **(B)**, Conidia **(C)**. A colony of the fungus on PDA medium observed from the upper side **(D)** from the underside **(E)**. Scale bars B and C 10 µm

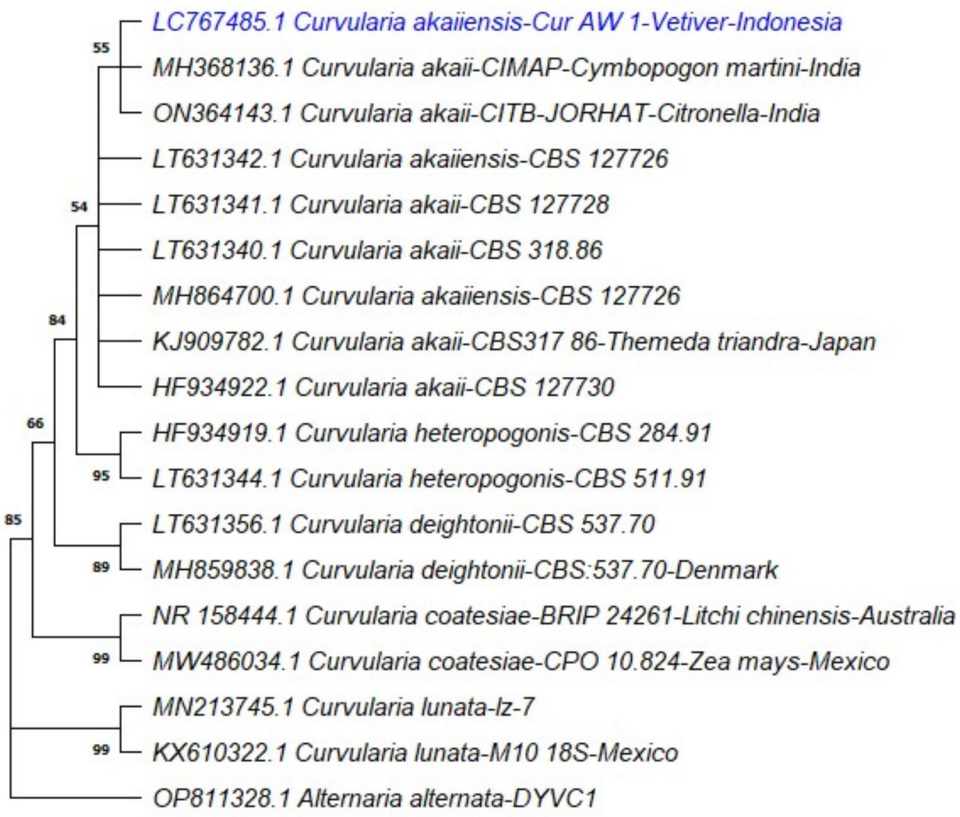

Fig. 2 Phylogeny tree of *C. akaiiensis* from vetiver in Indonesia with isolates from GenBank. *Alternaria alternata* isolate were used as outgroup

sp. genus and closely resembles *Curvularia akaiiensis*. The species is distinguished from other species primarily based on its morphology, particularly the spore size and shape. The sexual state was not detected from the fungal colony grown on PDA.

Further analysis was conducted using PCR for molecular identification. DNA extraction followed the method of Doyle et al. (1991). DNA amplification was performed using universal primers ITS 5 and ITS 4. The PCR program included an initial pre-denaturation cycle at 95 °C for 3 min, followed by 30 cycles of denaturation at 94 °C for 30 s, annealing at 55 °C for 20 s, extension at 72 °C for 1 min, and a final extension step at 72 °C for 7 min (Noveriza et al. 2020). The sequencing was conducted at First Base (Malaysia). The sequences obtained were edited, aligned, and analysed for similarity with related species from GenBank using BioEdit software. A phylogenetic tree was constructed using MEGA XI software, employing the Maximum-Likelihood tree with the General Time Reversible (GTR + G) model.

The fungal PCR results were sequenced, and the nucleotide sequences obtained were identical to *Curvularia akaiiensis* (GenBank: LC767485). The sequences showed a homology of 99.51%. The phylogenetic tree (Fig. 2) indicated that the *C. akaiiensis* isolate from vetiver belongs to the same group as the Indian strains CIMAP (MH368136) and CITB (ON364143) of *C. akaii*.

To assess pathogenicity, vetiver plants were separated into five groups, each consisting of three plants. A conidial suspension of 10^5 conidia/ml was sprayed onto the plant surfaces until runoff occurred. Approximately 50 ml of the conidial suspension was sprayed onto each plant. The inoculated plants were then covered with transparent plastic bags. Vetiver plants were also sprayed with sterilised distilled water, as the control. After 2 weeks, the inoculated plants exhibited characteristic symptoms similar to those observed in the field, while no symptoms were observed in the control plants. The same fungus, *C. akaiiensis*, was consistently re-isolated from the infected vetiver plants, confirming its role as the causal agent of the leaf spot disease. The pathogenicity test results support the association between *C. akaiiensis* and the disease occurrence. This is the first documented report of vetiver leaf spot disease caused by *C. akaiiensis* in Indonesia.

C. akaiiensis is classified within the Pleosporaceae family and is known to be a homothallic species according to Manamgoda et al. (2011). There is currently limited knowledge concerning the specific characteristics and ecological aspects of *C. akaiiensis*, and there have been no reports of this species since the initial description by Sivanesan in 1987. Based on our observatios, leaf spot disease occur throughout the year in traditional vetiver cultivation. The present study indicates a high degree of similarity between

C akaiiensis and *C akaii* in the ITS region. However, the conidial size of the present fungus was smaller than that of *C akaii* described by Sivanesan (1987). Zhang et al. (2020) also identified *C. akaiiensis* as a sister clade of *C. akaii* based on ITS, GAPDH and *trf*1 sequences. This research contributes to the identification of this specific disease among other leaf spot-like diseases and aids in the development of effective management strategies.

Acknowledgements The National Research and Innovation Agency provides support for research facilities and other technical assistance.

Declarations

Conflict of interest The authors have no conflicts of interest to declare that are relevant to this article.

References

Banerjee R, Goswami P, Pathak K, Mukherjee A (2016) Vetiver grass: an environment clean-up tool for heavy metal contaminated iron ore mine-soil. Ecol Eng 90:25–34. https://doi.org/10.1016/j.ecoleng.2016.01.027

Chomnunti P, Hongsanan S, Aguirre-Hudson B, Tian Q, Persoh D, Dhami MK, Alias AS, Xu J, Liu X, Stadler M, Hyde KD (2014) The sooty moulds. Fungal Divers 66(1):1–36. https://doi.org/10.1007/s13225-014-0278-5

Doyle J (1991) DNA protocols for plants. In: Hewitt GM, Johnston AWB, Young JPW (eds) Molecular Techniques in Taxonomy. NATO ASI Series, vol 57. Springer, Heidelberg (DE). https://doi.org/10.1007/978-3-642-83962-7_18

Grover M, Behl T, Virmani T, Bhatia S, Al-Harrasi A, Aleya L (2021) *Chrysopogon zizanioides*-a review on its pharmacognosy, chemical composition and pharmacological activities. Environ Sci Pollut Res Int 28(33):44667–44692. https://doi.org/10.1007/s11356-021-15145-1

Kobayashi T (1993) Diagnostic manual for industrial crop diseases in Indonesia. JICA-RISMCRI, Bogor (IND)

Manamgoda DS, Cai L, Bahkali AH, Chukeatirote E, Hyde KD (2011) *Cochliobolus*: an overview and current status of species. Fungal Divers 51(1):3–42. https://doi.org/10.1007/s13225-011-0139-4

Noveriza R, Rahajoeningsih S, Harni R, Miftakhurohmah NN (2020) Molecular identification of white root fungal pathogens and in vitro effect of nanopesticide. IOP Conf Ser Earth Environ Sci 428. https://doi.org/10.1088/1755-1315/418/1/012085

Semangun H (1992) Host index of plant diseases in Indonesia. Gadjah Mada University Press, Yogyakarta (IND)

Sivanesan A (1987) Graminicolous species of Bipolaris, Curvularia, Drechslera, Exserohilum and their teleomorphs. Mycological Papers 158:1–261

Zhang Q, Yang Z, Cheng W, Wijayawardene NN, Hyde KD, Chen Z, Wang Y (2020) Disease of *Cymbopogon citratus* (Poaceae) in China: *Curvularia nanningensis* sp nov. Mycokeys 63:49–67. https://doi.org/10.3897/mycokeys.63.49264