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# First Report of Basal Stem Rot Caused by *Ganoderma ryvardenii* on Oil Palm in Benin Republic

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Oil palm (*Elaeis guineensis*) is one of the main cash crops in emerging countries. Basal stem rot caused by *Ganoderma* spp. is a major disease in South-East Asia, with a disease incidence reaching 30%–50%, and up to 50% yield loss from 80% of 13-year-




**FIGURE 1** | Basal stem rot symptoms on adult palms in plantation with (a) foliar symptoms, (b) stem hole and (c) basidiomata at the lower stem.

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**FIGURE 2** | Colony morphology of BJ.Po.10 isolate after 10 days' incubation on potato dextrose agar at 28°C (isolate code BJ011 was used by the Agricultural Research Center of Perennial Plants to refer to the 2.BJ.Po.10 isolate).

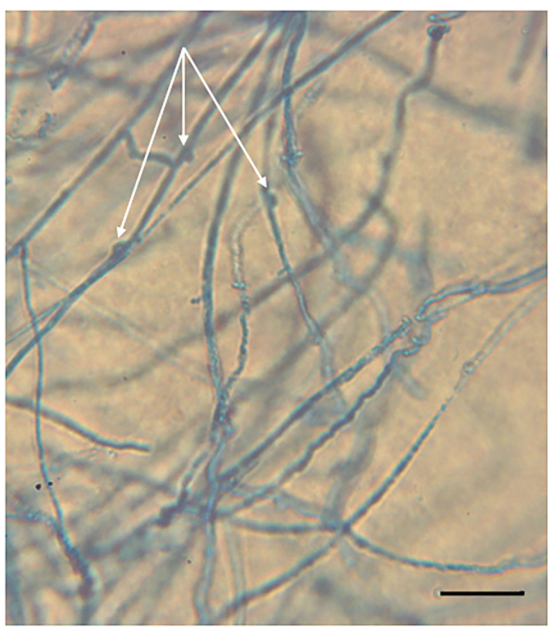


**FIGURE 3** | Microscopic observation of hyphae, white arrows indicate clamp connections. Bar = 20 μm.

old plantings in Malaysia (Flood et al. 2022). The disease has also been reported in several countries in Africa (Turner 1981).

For several years, many palm trees have shown typical symptoms of basal stem rot in the Benin Republic (mean incidence 2.1%) and particularly in the Pobè research station, in the Littoral province (6°59'17.0"N, 2°40'36.0"E). These symptoms are shorter and chlorotic new leaves, dead fronds hanging down around the trunk, and accumulation of unopened spear leaves, as well as holes and basidiomata that develop on the bole and lower trunk (Turner 1981; Corley and Tinker 2003) (Figure 1).

We collected 39 basidiomata from diseased palms growing in the Agricultural Research Center of Perennial Plants in Pobè, part of the National Agricultural Research Institute of Benin (Figure 1), cultured small pieces of dikaryotic tissue on water agar medium amended with streptomycin sulphate (0.5 g/L) and chloramphenicol (0.5 g/L), and then subcultured on potato dextrose agar (PDA) medium containing chloramphenicol (0.5 g/L). Nine isolates were obtained and one isolate, 2.BJ.Po.10, was selected for morphological description, molecular analysis and to test pathogenicity. Mycelium on PDA had a white cottony aspect (Figure 2). Hyphae were hyaline, about 2 μm thick in the centre and thinner on the margin, with septa-bearing clamp connections (Figure 3).

The pathogenicity of isolate 2.BJ.Po.10 was tested using a pot assay. One hundred 6-week-old oil palm seedlings were inoculated by depositing at each bole base, 20 mL of liquid inoculum (mixed mycelium in water) at a concentration of 50 mg/mL of fresh mixed mycelium. Twenty seedlings inoculated with 20 mL of tap water were used as control. Three weeks after inoculation, external symptoms appeared, characterized by necrotic spots on leaves, then fully necrotic leaves leading to the plant death. Internal symptoms, characterized by the rot of pseudobulbs, were revealed by longitudinal section. No symptoms were observed on control seedlings (Figure 4). The same fungus was reisolated from colonised tissue of infected seedlings but not from control ones, fulfilling Koch's postulates.

Total DNA was extracted from mycelium cultured on PDA. The ITS1-5.8S-ITS2 sequence region was amplified with ITS1-F / ITS4-B primers (Gardes and Bruns 1993), A BLASTn search showed that the resulting 541-nucleotide sequence had 98.6% identity and 100% coverage with *G. ryvardenni* holotype isolate HKAS 58053 (Kinge and Mih 2011). A phylogenetic analysis performed on several *Ganoderma* species taken from Zhou et al. (2015), confirmed that 2.BJ.Po.10 belonged to a well-supported monophyletic group containing only the whole *G. ryvardenni* isolates (PP = 0.91) (Figure 5).

To our knowledge, this is the first report of oil palm basal stem rot disease caused by *Ganoderma ryvardenii* in Benin Republic. Although the current incidence remains relatively low, the presence of *Ganoderma ryvardenii* in Benin represents critical information, as the incidence of this pathogenic fungus has increased considerably over the past 20 years in other African countries where it has been reported.



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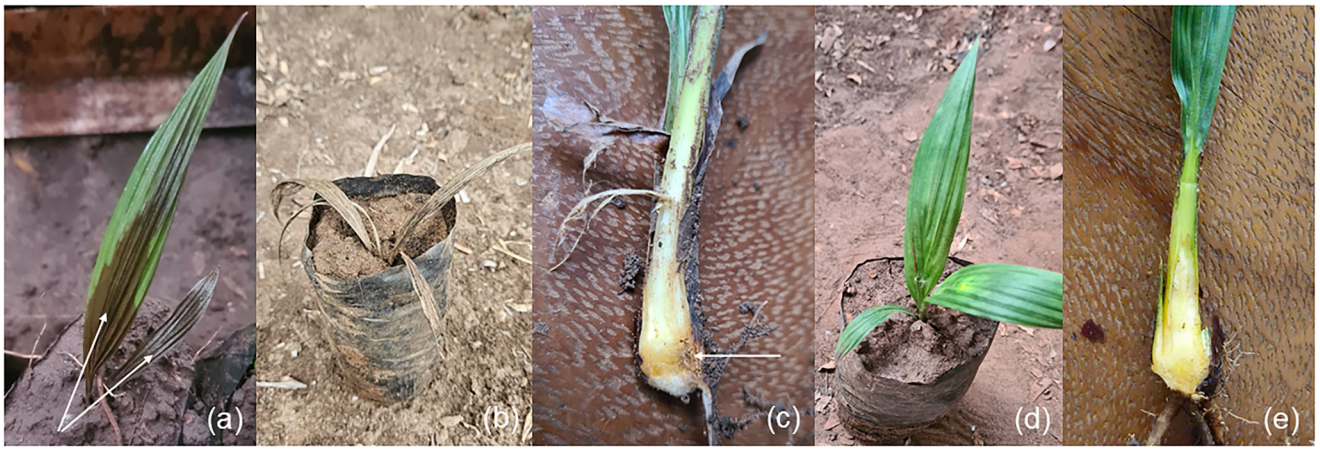



**FIGURE 4** | Disease symptoms on plantlets: (a) external leaf symptoms, (b) dead plantlet and (c) internal symptoms on inoculated seedlings, 3 weeks after inoculation (white arrows indicate necrotic areas on leaves and rotten tissues inside the pseudobulb); absence of both (d) external and (e) internal symptoms on healthy seedlings (non-inoculated controls).

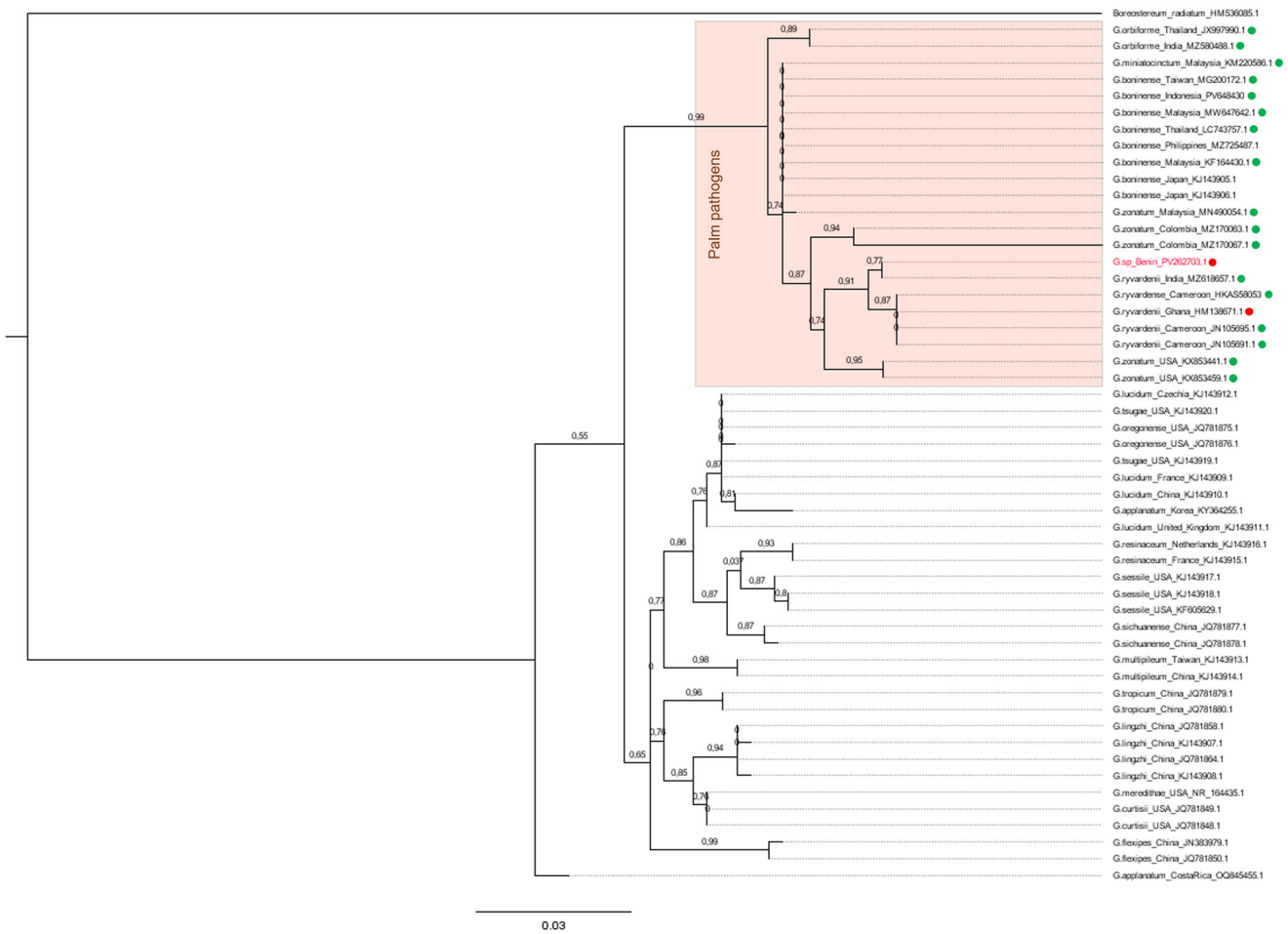



**FIGURE 5** | Phylogenetic tree showing the evolutionary relatedness of 2.BJ.Po.10 (PV262703) isolate amongst representative *Ganoderma* species (Zhou et al. 2015) based on the ITS sequence. Sequences were processed on "phylogeny.fr" website according the "one click" mode (aligned with MUSCLE v3.8.31, phylogeny estimated with PhyML 3.1/3.0). The tree was rooted using an outgroup species: *Boreostereum radiatum* (HM536085). The tree was visualized using FigTree v1.4.4. Green dots indicate specimens collected on palm trees, and red dots stand for specimens collected on palm trees for which Koch's postulates were validated.



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