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Black rot caused by *Phytophthora nicotianae* and *Phytophthora palmivora* on *Cattleya wittigiana* and *Dendrobium thyrsiflorum* in Brazil

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Abstract

Orchids are among the most cultivated flowers in the world. Plants of *Dendrobium thyrsiflorum* (*DT*) and *Cattleya wittigiana* (*CW*), cultivated at the Botanic Garden of Bauru in São Paulo, Brazil, presented black rot symptoms. The causal organism was identified as *P. palmivora* on *DT* and *P. nicotianae* on *CW* orchids based on its morphological and molecular characterization. Inoculation caused disease symptoms and Koch's postulates were fulfilled by re-isolation of the pathogen.

Keywords Orchid diseases · Oomycete · Diagnostics · Ornamentals

Orchids are economically valuable ornamental flowering plants, exhibiting significant diversity in flower size, shape, and color. There are approximately 29,524 species (Govaerts et al. 2022), and around 125,000 hybrid orchids registered worldwide (Jangyukala and Hemanta 2021). Common commercially cultivated include for example, *Cattleya*, *Cymbidium*, *Dendrobium*, *Epidendrum*, *Phalaenopsis* and *Vanda* (Tao et al. 2011). *Dendrobium thyrsiflorum* (*DT*), originate probably from Myanmar and Laos, stands out for its medicinal use and as an ornamental plant because of its graceful flowers (Yuan et al. 2011). *Cattleya wittigiana* (*CW*), originate from Atlantic forest of Brazil, have distinct broadly ovate leaves and flowers deep to light pink with a prominently scape longer than the leaf and petals bluntly obovate to subacute (Fowlie 1987). Orchid cultivation is challenged by several diseases, such as black rot caused by species of *Phytophthora* and *Pythium*. Symptoms of black rot can be seen as small lesions on leaves, pseudobulbs or roots. As the lesions age, they enlarge and may engulf the entire pseudobulb and leaf. The pathogen can spread through the rhizome to other portions of the plant. Eventually, the entire plant will be killed (Cating et al. 2009). Several *Phytophthora* species have been recorded to cause significant economic losses globally by infecting orchids (Uchida 1994; Orlikowski and Szkuta 2006; Cating et al. 2009).

Dendrobium thyrsiflorum and *CW* are among the orchid species cultivated at the Botanic Garden of Bauru in São Paulo, Brazil. In March 2020 and May 2023, 22% ($n = 11$) and 10% ($n = 1060$) of *DT* and *CW* plants presented leaves with characteristic black rot symptoms caused by *Phytophthora* spp. (Figure 1A and C). In addition to the dark, water-soaked lesions on the leaves, on *CW* plants the symptoms progressed quickly (< 1 week) to bulb rot and plant death. Symptomatic leaf tissues were disinfected (70% ethanol for 30 s, 1% sodium hypochlorite for 2 min) and placed on potato dextrose agar (PDA). After seven days at 25 ºC, five isolates were obtained from *DT* (*DT*1-5) and four from *CW* (*CW*1-4), from hyphal tips. The isolates produced papillate sporangia, ovoid to ellipsoid and with a short pedicel. Total DNA of isolates *CW*2, *DT*2, and *DT*4 was extracted using the cetyl trimethyl ammonium bromide method. Sequences of the translation elongation factor 1α (EF1A), β-tubulin (β-TUB) and internal transcribed spacer (ITS) (Liu et al. 2023; Yang et al. 2017) were amplified with EF1-1018 F/EF1-1620R, TUBUF2/

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Fig. 1 Black rot caused by *Phytophthora* spp. in orchids in the plant collection of the Botanical Garden of Bauru. *Phytophthora nicotianae* symptoms on *Cattleya wittigiana* in the field **(A)** and from the pathogenicity assay **(B)**.*Phytopthtora palmivora* symptoms on *Dendrobium thyrisiflorum* in the field **(C)** and from the pathogenicity assay **(D)**

TUBUr1 and ITS1/ITS4 primer pairs (Kroon et al. 2004; Stielow et al. 2015; White et al. 1990), sequenced and deposited in GenBank (accession numbers PP068465, PP067963 and PP067964 for ITS, PP092935, PP092936 and PP092937 for β-TUB and PP092938, PP092939 and PP092940 for EF1A). Isolate *CW*2 clustered with *P. nicotianae* strain (MYA-4039), while isolates *DT*2 and *DT*4 clustered with *P. palmivora* strain (MYA-4037) with the Bayesian Posterior Probability of 99.9 (Fig. 2). The isolates *CW*2, *DT*2, and *DT*4 were deposited in at the Fungal Culture Collection "Mario Barreto Figueiredo" of the Agency for Agribusiness Technology (APTA), São Paulo Biological Institute, Brazil (MMBF) as MMBF 01/24, MMBF 02/24 and MMBF 03/24, respectively.

Healthy plants of *DT* and *CW* orchids were inoculated with five *P. palmivora* isolates and four *P. nicotianae* isolates, respectively, using three leaves per isolate. A mycelial disc (0.5 mm) was deposited on a wound made with a hypodermic needle. PDA discs were used as negative control and for *DT*, inoculation with a mycelial disc without leaf injury was also adopted. The plants were kept in a greenhouse under a humid chamber for the first 24 h, and between 4 and 7 days for *DT* and 4–5 days for *CW*, symptoms were observed in 87% and 92% of inoculated leaves,

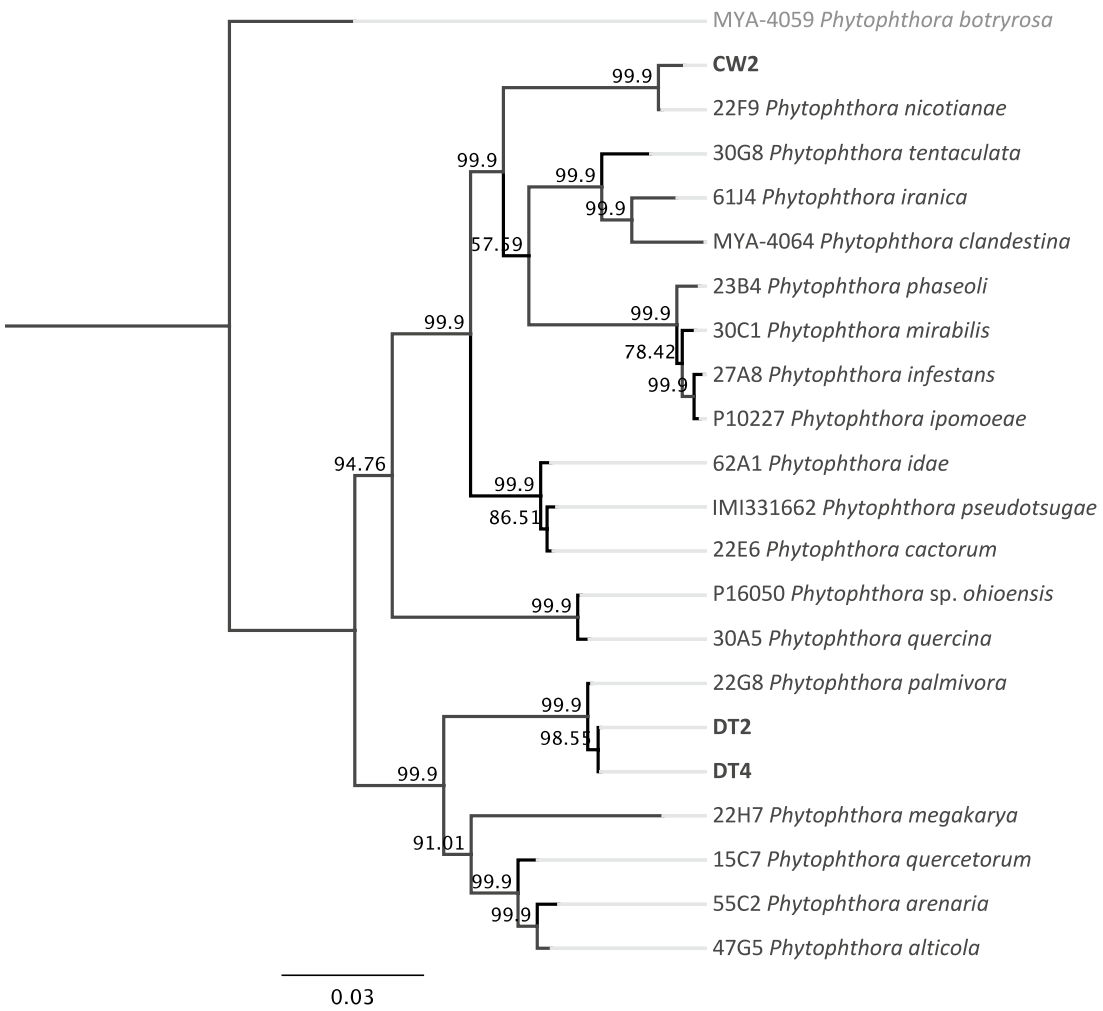

Fig. 2 Bayesian inference phylogenetic tree reconstructed from the combined TUB2, TEF1-alpha and ITS sequence alignment of *Phytophthora* spp. strains. *Phytophthora nicotianae* strain (CW2), isolated from *Cattleya wittigiana*, and *Phytophthora palmivora* strains (DT2 and DT4) isolated from *Dendrobium thyrsiflorum* are emphasized in bold. *Phytophthora botryrosa* was used as outgroup. Bayesian posterior probability (BPP) are shown at the nodes. The scale bar represents the number of expected changes per site

respectively. In inoculation without wounding, symptoms were observed in only 27% of the leaves. Mock inoculated plants remained asymptomatic. *Phytophthora* spp. were reisolated from all symptomatic plants (Fig. 1B and D), fulfilling Koch's postulate.

Phytophthora palmivora has been reported in *Dendrobium* species in several countries, such as *Dendrobium bigibbum* in Japan (Rahman et al. 2014), D. *crumenatum* in Indonesia (Schwarz 1927), D. *phalaenopsis* in Japan (Masanto et al. 2019), D. *maccarthiae* in Sri Lanka and United States (Erwin and Ribeiro 1996) and *Dendrobium* sp. in Brunei and United States (Peregrine and Ahmad 1982; Oudemans and Coffey 1991), while *P. nicotianae* has been reported on *Cattleya* sp. in Taiwan, United States (Erwin and Ribeiro 1996), and Australia (Cook and Dube 1989), without, however, the authors identifying the species of *Cattleya*. *Phytophthora nicotianae* has also been reported on *DT* in China (Tao et al. 2011). According to the USDA fungal database (https://nt.ars-grin.gov/fungaldatabases/fungushost/fungushost.cfm) and other available literature (Bag et al. 2024), no official report is available for *P. palmivora* and *P. nicotianae* causing disease on *DT* and *CW* orquids, respectively, in the world. The correct identification of *Phytophthora* spp. associated with the disease is crucial for the development of correct disease management strategies.

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Author contributions All authors contributed to the study conception and design. Conceptualization, methodology, formal analysis and investigation, and writing were performed by Thaís Regina Boufleur, Priscila Yukari Takaki Ino, Nelson Sidnei Massola Júnior and Ivan Herman Fischer. Material preparation and data collection were performed by Viviane Camila de Oliveira and Luiz Carlos de Almeida Neto. All authors read and approved the final manuscript.

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Data availability We state that the sequences of the regions used for *Phytophthora*spp. were deposited at GenBank and the accession number were provided in the main manuscript. The sequences will be released by GenBank upon publication of this manuscript, therefore, the flat files of the submission are available for the reviewers in the supplementary material.

Declarations

Ethical approval Not Applicable.

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

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