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Technical Article

Soft rot on the stems of *Zamioculcas zamiifolia* caused by *Sclerotium rolfsii*

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Abstract

Zamioculcas zamiifolia (Araceae) is one of the most widely grown exotic species in Brazil as ornamental plants and in landscape design. Despite tolerating transport and being well adapted to low-light environments, this ornamental is attacked by different pathogens. Thus, the aim was to detect and identify the pathogen that causes stem rot in commercial *Z. zamiifolia* crops. *Z. zamiifolia* plants exhibiting stem rot symptoms were sent for phytosanitary diagnosis. In a culture medium, the fungal isolate obtained (SR-001) displayed the following morphological characteristics: cotton-like aerial mycelium, septate hyaline hyphae with no spore production, and the formation of small brown spherical sclerotia. To confirm pathogenicity, *Z. zamiifolia* plants were inoculated with the SR-001 isolate and, after fifteen days, the fungus was re-isolated when the same rot symptoms emerged. The SR-001 isolate was identified as *Sclerotium rolfsii* and its representative sequence was deposited in GenBank (Access MG694322). This fungal isolate has not been associated with diseases in *Z. zamiifolia* in Brazil, and this is the first report of the fungus infecting this ornamental plant species in a cultivated area.

Keywords: *Sclerotium rolfsii*, Damping-off, ornamental plants, sclerotium rot.

Resumo

Podridão da haste de *Zamioculcas zamiifolia* causada por *Sclerotium rolfsii*

A espécie *Zamioculcas zamiifolia* (Araceae) está entre as espécies exóticas mais utilizadas e cultivadas no Brasil como plantas ornamentais e requisitada em projetos de paisagismo. Apesar de apresentar durabilidade no transporte e adaptação em ambientes com pouca luz, esta ornamental é acometida por diferentes patógenos. Assim o objetivo foi detectar e identificar o patógeno causador de podridão na haste de *Z. zamiifolia* em cultivos comerciais. Plantas de *Z. zamiifolia* apresentando sintomas de podridão nas hastes foram encaminhadas para diagnose fitossanitária. O isolado fúngico obtido, SR-001, apresentou, em meio de cultura, características morfológicas de micélio aéreo de aspecto cotonoso, hifas hialinas septadas, sem produção de esporos, e com formação de pequenos escleródios esféricos de coloração marrom. Para comprovar a patogenicidade, plantas de *Z. zamiifolia* foram inoculadas com o isolado SR-001 e, após quinze dias, procedeu-se o reisolamento do fungo quando do aparecimento dos mesmos sintomas de podridão. O isolado SR-001 foi identificado como *Sclerotium rolfsii* e a sequência representativa de *S. rolfsii* foi depositada no GenBank (Acesso MG694322). Este isolado fúngico não tem sido associado com doenças em *Z. zamiifolia* no Brasil, sendo assim este o primeiro relato do fungo infectando essa espécie de planta ornamental em área de cultivo.

Palavras-chave: *Sclerotium rolfsii*, Damping-off, plantas ornamentais, podridão-de-esclerotium

Introduction

Growing plant species for ornamental purposes is a promising sector of Brazilian agriculture, primarily among exotic species, which are the most sought after, mainly because of their beauty, adaptability and durability. *Zamioculcas zamiifolia*, of African origin, is widely used in landscape projects. This herbaceous plant from tropical regions belongs to the family Araceae, and grows to a height of 45-60 cm (Moullec et al., 2015). One of the main characteristics that has attracted the attention of producers is its good adaptability to low-light environments, in addition to exhibiting durability and tolerance to transport (Khaksar et al., 2017).

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Despite being resistant to physical damage, *Z. zamiifolia* plants are affected by biotic factors such as diseases, whose incidence has been increasing in productive settings. Pathogenic agents, particularly fungi and viruses that cause soft rot and mosaic, such as the soil fungus *Phytophthora nicotianae* (Sanahuja et al., 2016), leaf necrotic lesions fungus (Zhou e Li, 2017) and the virus *Konjac mosaic virus* (KoMV) (Alexandre et al., 2013), are among the main phytosanitary problems that cause damage, resulting primarily from dissemination via matrix planting.

Due to the restricted information on diseases associated with *Zamioculcas zamiifolia* plants, and given the importance of correctly diagnosing ornamental plant diseases to promote understanding of disease management, this study aimed to detect and identify possible phytopathogenic agents associated with the symptoms observed in *Z. zamiifolia* plants.

Material and Methods

Obtaining the isolate

Cultivated *Zamioculcas zamiifolia* plants, with symptoms of soft rot on the stems and yellowing leaves, were sent for phytosanitary analysis. Lesion fragments were removed from the infected tissues. After surface disinfection with 2% NaClO (2 min) and washing in sterile distilled water, the fragments were deposited at equidistant points, in triplicate, on Petri dishes containing potato dextrose agar (PDA) medium and incubated in a growth chamber at a temperature of 28 ^{o}C and 12-hour photoperiod (Nandi et al., 2017). After three days, 5 mm-wide mycelium disks were removed from the ends of the growing mycelium, originated from the fragments, and transferred to the center of the 90-mm Petri dishes (in triplicate) containing PDA to form a pure culture.

Morphological and molecular identification

The isolate obtained by the isolation method was morphologically characterized. Characteristics such as color and texture of the colonies, type of hyphae, as well as reproductive structure formation and resistance, were analyzed. Following morphological identification, genomic DNA was extracted from the pure culture of the isolate using the CTAB 2X method (Boiteux et al., 1999). The total DNA extracted was used to amplify the ITS (Internal Transcribed Spacer) region by PCR (polymerase chain reaction). The reaction consisted of 100 µM of deoxynucleotide triphosphates, 0.1 µM ITS1 primer (5′TCC GTA GGT GAA CCT GCG G 3′) and ITS4 (5′TCC TCC GCT TAT TGA TAT GC 3’) (White et al., 1990), 1x PCR buffer with 2.0 mM of $MgCl_2$, 2 µL of DNA sample at a concentration of 30ηg.µL^{-1} and 1U of *Taq* polymerase in a final volume of 25 µL. Amplification was conducted with initial denaturation at 96 °C for 10 minutes, followed by 30 cycles of 95 °C for 1 minute, 60 °C for 1 minute, 72 °C for 1 minute and a final extension at 72 °C for 10 minutes. After assessing amplification by 1% agarose gel electrophoresis, the PCR product was purified using the AxyPrep™ DNA Gel Extraction Kit (Axygen), and sent for sequencing. The sequences obtained were analyzed with BioEdit (Hall, 1999) and compared with the NCBI (National Center for Biotechnology Information) GenBank using BLAST (Basic Local Alignment Search Tool).

Pathogenecity test

To confirm pathogenicity of the isolate obtained, two inoculation methods were applied to healthy *Z. zamiifolia* plants grown in pots containing autoclaved substrate. In the first method, mycelium disks (5 mm diameter) from the isolate grown for 15 days on PDA medium were attached to the base of the stem and on the leaves of the plant, with and without injury, and maintained in a humidity chamber for 48 hours at ambient temperature. Control plants consisted only of PDA medium disks with and without the presence of fungal propagules. For the second inoculation method, carried out by soil infestation with sclerotia from the isolate produced in culture medium, around 100 sclerotia were mixed at a soil depth of 0-10 cm in pots containing 1.0 L of autoclaved substrate, and *Z. zamiifolia* seedlings were planted. Four repetitions were used for both pathogenicity tests. After inoculation, plants were maintained in a regime of constant irrigation at ambient temperature. The plants were monitored to check for symptoms and were re-isolated.

Results and Discussion

Morphological and molecular identification

A fungal microorganism was obtained from fragments of plants in PDA medium (SR-001 isolate), which, after 15 days of incubation in pure culture at ambient temperature, exhibited a white cotton-like aerial mycelium, with septate hyaline hyphae, and no asexual spore production, also observed by others authors (Mahadevakumar et al., 2016; Park et al., 2018). The formation of 50 small dark brown spherical sclerotia (1.3 ± 0.25 mm in diameter) was observed, totaling around 210 sclerotia produced in Petri dishes (90 mm in diameter). The characteristics that differentiate *S. rolfsii* from *S. delphinii* include the cotton-like nature of the aerial mycelium in PDA culture medium, abundance of sclerotia (200 to 350/plate) and small size (1-2 mm) when compared with *S. delphinii*, which produces 20 to 30 sclerotia/plate, measuring 3 to 5 mm in diameter (Mahadevakumar et al., 2016). Based on these morphological characteristics, the SR-001 isolate was identified as *Sclerotium rolfsii* Sacc (Teleomorph: *Atelia rolfsii*) (Punja e Damiani, 1996) (Figure 1A).

Amplification and sequencing of the ITS1/ITS4 region of the SR-001 isolate confirmed *S. rolfsii* as the phytopathogenic agent. The sequence obtained showed 96% similarity with the SrGhMKS2 isolate (GenBank access no. KP412272.1) from *Athelia rolfsii*, which is the sexual phase of *S. rolfsii*. The sequence that represents the SR-001 fungal isolate identified as *S. rolfsii* was deposited in GenBank (access no. MG694322).

Pathogenecity test

With respect to the pathogenicity test, after 15 days' inoculation, the same rot symptoms exhibited by the samples from which the fungus was isolated were observed in both the leaves and stems of the plants inoculated with the SR-001 isolate. In mycelium disk inoculation, only uninjured leaf tissues displayed no symptoms. This is likely due to the fungus' low efficiency in directly colonizing aerial tissues, given that its natural infection is more associated with root and stem rot, an environment characterized by climatic conditions favorable to plant cell infection by the fungus. Results of inoculation by soil infestation with sclerotia show that the pathogen's capacity to infect the plant develops under natural soil moisture and temperature conditions, producing symptoms on the plant stem (Figure 1B).

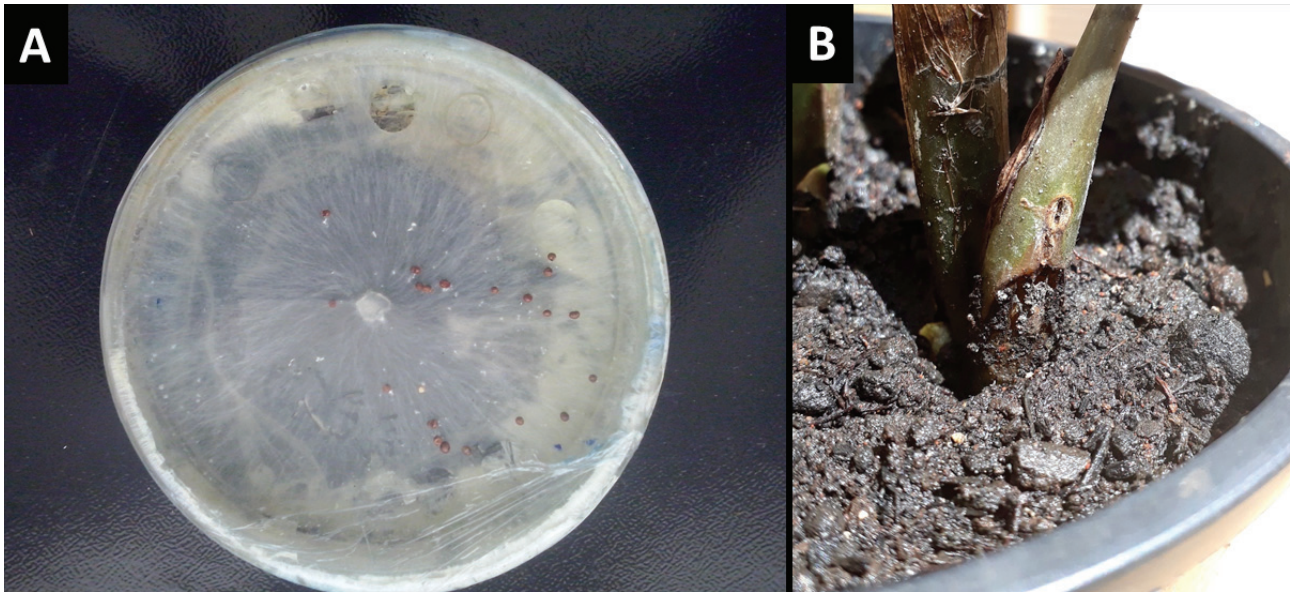

Figure 1. A) *Sclerotium rolfsii* in PDA medium; B) Symptom of rot on the stem of a *Zamioculcas zamiifolia* seedling 15 days after inoculation of *S. rolfsii* by sclerotia soil infestation.

In symptomatic tissues, fragments were once again used to isolate the pathogen, confirming it to be *Sclerotium rolfsii*, a *sine qua non* requirement to complete Koch's postulates. The fungus *S. rolfsii*, considered an aggressive, difficult-to-control phytopathogen, has been reported in more than 500 plant species, including dicotyledons and monocotyledons (Mahadevakumar et al., 2015; Mahadevakumar et al., 2018). It causes the disease known as sclerotium rot or sclerotium wilt, widely distributed in tropical and subtropical regions (Punja and Damiani, 1996; Mahadevakumar et al., 2016). *S rolfsii* is known to infect several economically important crops in various stages of their growth and development, in addition to producing survival structures (Mahadevakumar et al., 2016; Paul et al., 2017; Shrestha et al., 2018). Studies conducted in Sri Lanka (Jegathambiga et al., 2010) reported the occurrence of rot symptoms in *Zamioculcas* sp. plants caused by *S. rolfsii* as the primary cause of the decline in plant exports in the country.

Conclusion

The fungus *Sclerotium rolfsii* is associated with diseases in *Z. zamiifolia*. As such, this is the first report of soft stem rot caused by *S. rolfsii* occurring naturally in the cultivated areas of this ornamental species in Brazil.

Author Contribution

M.C.S.[0000-0001-9948-5789]: experiment conduction and manuscript writing. **R.C.F.**[0000-0002-9537-1503]: planning, manuscript review, experiment conduction and molecular analysis. **G.A.R**[0000-0001-8167-0652]: revision of the manuscript, molecular analysis. **E.C.D.**[0000-0001-7009-2041]: orientation, revision of manuscripts. **M.G.C.**[0000-0001-8338-4107]: orientation, planning and revision of manuscripts.

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