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First report of *Paraphaeosphaeria angularis* as endophyte in sugarcane (*Saccharum officinarum*) from India

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Brazil and India put together contribute to more than 50% of global sugarcane (*Saccharum officinarum* L.) production. This important crop is known to have multiple utilities including production of molasses, sugar and ethanol. Thus, studying microbial diversity, especially endophytes that are known to contribute to plant fitness to overcome biotic and abiotic stresses become imperative. Studies on endophytic fungi of sugarcane include that of Srivastava *et al*. (2017) from India, de Souza *et al*. (2016), Romão-Dumaresq *et al*. (2016) and Fors *et al*. (2020) from Brazil, Khunnamwong *et al*. (2014) from Thailand, and Lufeng *et al*. (2019) from China. Since there are limited studies on endophytes of sugarcane (Rashmi *et al*. 2019), present study is embarked on screening sugarcane leaf for the presence of fungal endophytes.

Studies on fungal endophytes of any plant host from any geographical region invariably result in isolation of sterile forms as fungal endophytes. In order to induce sporulation in these sterile mycelia, various methods have been followed that include growing them on media containing plant extracts and incubating them under different conditions (Taylor *et al*. 1999, Guo *et al*. 2000), but in spite of these, only few of the isolates sporulate. Therefore, molecular methods have become essential for the identification of these sterile endophytic fungi (Wang *et al*. 2005). The present work on isolation of endophytic fungi from sugarcane leaves resulted in recording a number of sterile forms as endophytes. In this study, the presence of *Paraphaeosphaeria angularis* (*Dothideomycetes, Pleosporales, Didymosphaeriaceae*), isolated as a sterile form from sugarcane, based on sequence analysis of 5.8S gene and ITS regions of rDNA is reported. This is the first report of this fungus as sugarcane leaf endophyte from India, while other species belonging to this genus have been reported as leaf spot pathogens from the host.

Fresh leaves were collected from sugarcane hosts growing in Puducherry, India, on March 18, 2022. The leaves were washed in tap water before surface sterilisation. From the middle of the lamina, small segments measuring approximately 0.5 cm^2 in size were obtained and surface sterilized by a four-step sterilization protocol (Stuart *et al.* 2010). The leaf bits were first immersed in 50% ethanol for 1 min, then in 4% NaOCl for 1.5 min, then in 50% ethanol for 0.5 min and finally rinsed in sterile distilled water. The surface sterilised bits were inoculated in 9 cm diameter. Petri-plates containing potato dextrose agar (PDA) were added with antibiotic (Chloramphenicol 150 mg/litre). The plates were observed for a period of 4 weeks for endophyte growth.

DNA isolation and PCR analysis: DNA extraction from the endophyte was done following Gardes and Bruns (1993). The PCR amplification of the ITS1-5.8S-ITS2 segment was performed with the ITS1 and ITS4 primers (White *et al*. 1990). The PCR reaction was performed in a thermal cycler (GeneAmp PCR System 9700, Applied Biosystems) with the following conditions: 98°C for 30 sec, thermal cycling for 40 cycles (98°C, 58°C, and 72°C for 5, 10 and 15 sec respectively), and finally at 72°C for 60 sec. The amplified product was sequenced and deposited in GenBank database with the accession number OP125755.

Phylogenetic analysis: The ITS sequences of the fungal endophyte was taken up for phylogenetic analysis. A preliminary blast analysis was performed and based on the sequence similarity, a total of 14 nucleotide sequences were selected for further analysis. A multiple sequence alignment was performed using CLUSTALW and manually edited using MEGA11 software (Tamura *et al*. 2021). The Maximum Likelihood method based on the Tamura-Nei model (Tamura and Nei 1993) was used to infer the evolutionary history. A final tree was constructed with branch lengths measured as the number of substitutions per site based on the above parameters and 1000 bootstrap replicates.

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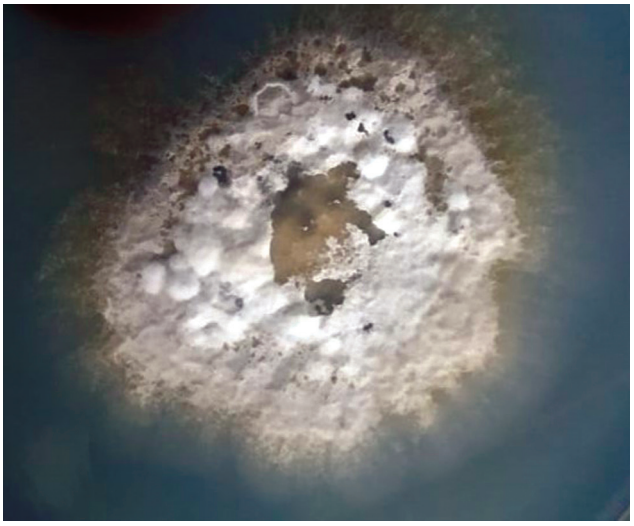

Fig 1 Colony morphology of *Paraphaeosphaeria angularis.*

A preliminary study on fungal endophytes of sugarcane resulted in recording 9 different sterile forms. Sequence analysis of 5.8S gene and ITS regions of rDNA for one of the sterile endophyte (Fig 1) resulted in assigning the taxa *Paraphaeosphaeria angularis* to the sterile form based on the evolutionary history as inferred using the Maximum Likelihood method and Tamura-Nei model (Fig 2). It is imperative to mention here that for distinguishing some of the taxa like *Paraphaeosphaeria,* morphological studies coupled with phylogenetic analysis are important (Ariyawansa *et al*. 2020). *Paraphaeosphaeria angularis* obtained in the present study was clustered with *P. angularis* (NR172222 and JX496013) (Fig 2) isolated from sugarcane grown in Brazil. *Paraphaeosphaeria angularis* has not been reported from sugarcane in India, but has been isolated from sugarcane in Brazil (Verkley *et al*. 2014), and recorded as novel species of *Paraphaeosphaeria*. Verkley *et al*. (2014) have described the conidial state of *P. angularis* with immersed mycelium, covered by a diffuse mat of pure white finely felted aerial mycelium that is similar in morphology to *P. angularis* in the present study.

Srivastava *et al*. (2017) studied fungal endophtes of sugarcane from India, but in both the studies *Paraphaeosphaeria* has not been recorded. Rashmi *et al*. (2019) reported 6 different species of *Paraphaeosphaeria* as endophytes from different host plant species but none from India. In a recent study, Silva *et al*. (2018) recorded *P. arecacearum* as an endophyte from *Paullinia cupana*, a multipurpose plant (Marques *et al*. 2019) from Brazil. A species belonging to the genus *Paraphaeosphaeria,* i.e. *P. michotii* has been reported as pathogen in sugarcane causing leaf spot (Nyvall 1989). Further, the genus *Paraphaeosphaeria* includes plant pathogens, biocontrol agents and endophytic fungi (Baroncelli *et al*. 2020). Along with some bacterial species, fungal species in the genus *Paraphaeosphaeria* is known to be either involved in or associated with beech leaf disease (Ewing *et al*. 2021, Fearer *et al*. 2022). Also, endophytic *Paraphaesphaeria sporulosa* isolated from *Paepalanthus planifolius* produced novel isoquinoline alkaloid (de Amorim *et al*. 2022). Thus, assigning taxa to sterile forms will reveal the presence of novel endophytes and fungi that have not been reported earlier from the host plants as in the case of present study, presence of pathogens that are latent and do not sporulate in culture media, and also fungi with bioactive potential.

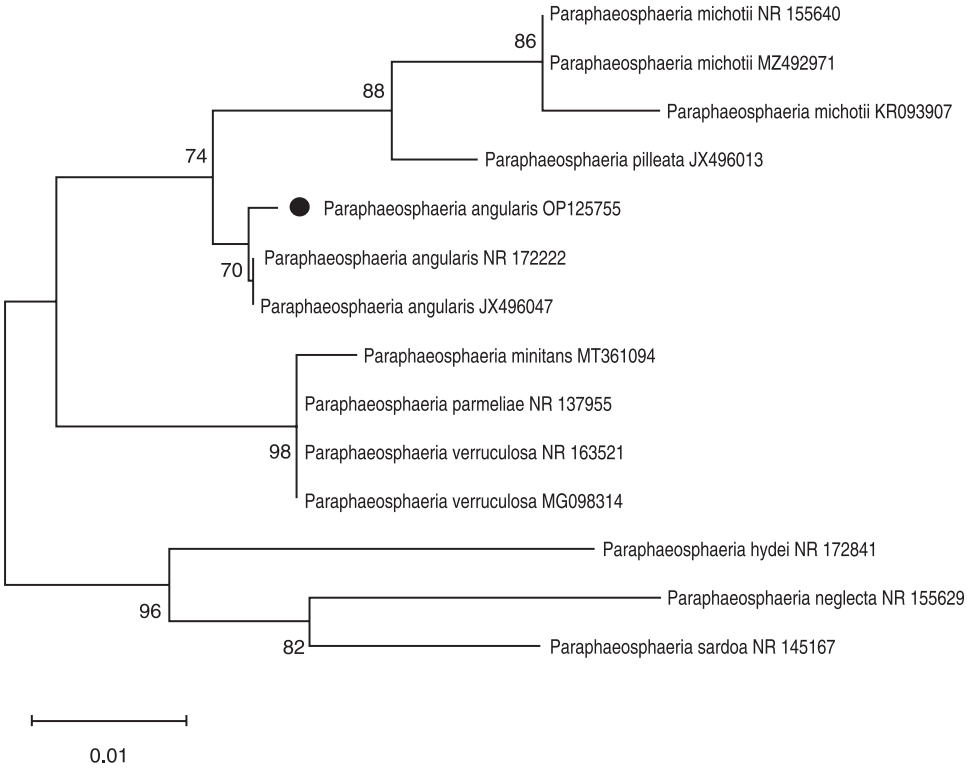

Fig 2 Maximum Likelihood tree of the internal transcribed spacer (ITS) region of *Paraphaeosphaeria angularis* isolated from sugarcane leaf (OP125755) and similar sequences from GenBank.

SUMMARY

Sugarcane (*Saccharum officinarum* L.) is considered to be an important crop that is utilized for production of molasses, sugar and ethanol. Thus, identifying microbes associated with this crop plant will give more insight into plant-microbe interaction. Further, there are limited studies on sugarcane fungal endophytes. Therefore, sugarcane leaves

obtained from approximately 3 month old sugarcane plants growing in Puducherry were screened for the presence of fungal endophytes, during March 2022. The study conducted to identify fungal endophytes of sugarcane resulted in isolation of a sterile form which was identified as *Paraphaeosphaeria angularis*, an ascomycetous fungal species, based on sequencing of the internal transcribed spacer and the 5.8S rDNA region. Maximum Likelihood method was used to infer the evolutionary history. It is the first report of this fungus, as endophyte, from sugarcane host from India. The genus *Paraphaeosphaeria* is known to include plant pathogens, biocontrol agents and endophytic fungi. This study highlights the importance of studying and assigning taxa to the sterile forms especially from crop plants, since a species belonging to *Paraphaeosphaeria,* viz. *P. michotii* is reported as a pathogen from sugarcane from other countries. Further studies will reveal the potential of *P. angularis* as fungal species known to occur just as an endophyte or a capable pathogen.

REFERENCES

Ariyawansa H A, Tsai I, Thambugala K M, Chuang W-Y, Lin S-R, Hozzein W N and Cheewangkoon R. 2020. Species diversity of Pleosporalean taxa associated with *Camellia sinensis* (L.) Kuntze in Taiwan. *Scientific Reports* **10**: 12762.

Baroncelli R, Da D, Lio G and Sarrocco S. 2020. Genome resources for the endophytic fungus *Paraphaeosphaeria sporulosa*. *Molecular Plant-Microbe Interactions* **33**: 1098–99.

de Amorim M R, Paz T A, Pinto L, Hilário F, Zanini C L, Aguiar A, Silva D, Furlan M, Guido R, Bauab T M, Netto A and Dos Santos L C. 2022. New isoquinoline alkaloids from *Paraphaeosphaeria sporulosa* F03, a fungal endophyte isolated from *Paepalanthus planifolius*. *Planta medica* **88**(12): 994–003.

de Souza R S C, Okura V K, Armanhi J S L, Jorrin B, Lozano N, da Silva M J, Gonzale-Guerrero M, de Araujo L M, Verza N C, Bagheri H C, Imperial J and Arruda P. 2016. Unlocking the bacterial and fungal communities assemblages of sugarcane microbiome. *Scientific Reports* **6**: 28774.

Ewing C J, Slot J, Benítez Ponce M-S, Rosa C, Malacrinò A, Bennett A and Bonello A. 2021. The foliar microbiome suggests fungal and bacterial agents may be involved in the beech leaf disease pathosystem. *Phytobiomes Journal* **5**: 335–49.

Fearer C J, Conrad A O, Marra R E, Georskey C, Villari C, Slot J and Bonello P. 2022. A combined approach for early in-field detection of beech leaf disease using near-infrared spectroscopy and machine learning. *Frontiers in Forest and Global Change* **5**: 934545.

Fors R O, Patreze C M, Louro Berbara R L, Carbone Carneiro M A and Saggin-Junior O J. 2020. Dark septate endophytic fungi associated with sugarcane plants cultivated in São Paulo, Brazil. *Diversity* **12**: 351.

Gardes M and Bruns T D. 1993. ITS primers with enhanced specificity for basidiomycetes application to the identification of mycorrhizae and rusts. *Molecular Ecology* **2**: 113–118.

Guo L D, Hyde K D and Liew E C Y. 2000. Taxonomic placement of fungal endophytes from *Livistona chinensis*. *(In) XVI International Botanical Congress*, St. Louis, USA, August 1–7, Abstract No. 3117.

Khunnamwong P, Surussawadee J, Jindamorakot S and Limtong S. 2014. *Wickerhamiella siamensis* f.a., sp. nov., an endophytic and epiphytic yeast species isolated from sugar cane leaf. *International Journal of Systematic and Evolutionary Microbiology* **64**: 3849–55.

Lufeng L I U, Haichun C U N, Pengfei H E, Yining D I, Yixin W U, Lilian H E, Fusheng L I and Yueqiu H E. 2019. Isolation, identification and multiple function analyses of sugarcane endophytes. *Chinese Journal of Tropical Crops* **40**: 1144–52.

Marques L L M, Ferreira E D F, de Paula M N, Klein T and de Mello J C P. 2019. *Paullinia cupana*: a multipurpose plant — a review. *Revista Brasileira de Farmacognosia* **29**: 77–10.

Nyvall R F. 1989. Diseases of Sugarcane. *Field Crop Diseases Handbook*. Springer, Boston, MA. https://doi.org/10.1007/978-1-4757-5221-2_20

Rashmi M, Kushveer J S and Sarma V V. 2019. A worldwide list of endophytic fungi with notes on ecology and diversity. *Mycosphere* **10**: 798–1079.

Romão-Dumaresq A S, Dourado M N, Fávaro L C dL, Mendes R, Ferreira A and Araújo, W L. 2016. Diversity of cultivated fungi associated with conventional and transgenic sugarcane and the interaction between endophytic *Trichoderma virens* and the host plant. *PLoS One* **11**: e0158974. doi:10.1371/journal.pone.0158974

Silva F A, Liotti R G, Boleti A, Reis É M, Passos M, Dos Santos E L, Sampaio O M, Januário A H, Branco C, Silva G, Mendonça E and Soares M A. 2018. Diversity of cultivable fungal endophytes in *Paullinia cupana* (Mart.) Ducke and bioactivity of their secondary metabolites. *PloS One* **13**(4): e0195874.

Srivastava S, Gupta P S, Lal S and Sinha O K. 2017. Rapid identification of endophytic fungi of sugarcane *Saccharum* spp. hybrid) using PCR-RFLP of rDNA. *Journal of Environmental Biology* **38**: 21–26.

Stuart R M, Romão A S, Pizzirani-Kleiner A A, Azevedo J L and Araújo W L. 2010. Culturable endophytic filamentous fungi from leaves of transgenic imidazolinone-tolerant sugarcane and its non-transgenic isolines. *Archives of Microbiology* **192**: 307–13.

Tamura K and Nei M. 1993. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Molecular Biology and Evolution* **10**: 512–26.

Tamura K, Stecher G and Kumar S. 2021. MEGA 11: Molecular Evolutionary Genetics Analysis Version 11. *Molecular Biology and Evolution* **38**(7): 3022–27.

Taylor J E, Hyde K D and Jones E B G. 1999. Endophytic fungi associated with the temperate palm, *Trachycarpus fortunei* within and outside its natural geographic range. *New Phytologist* **142**: 335–46.

Wang Y, Guo L D and Hyde K D. 2005. Taxonomic placement of sterile morphotypes of endophytic fungi from *Pinus tabulaeformis* (Pinaceae) in northeast China based on rDNA sequences. *Fungal Diversity* **20**: 235–60.

White T J, Bruns T, Lee S and Taylor J W. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *PCR Protocols*: *A Guide to Methods and Applications*, pp. 315–22. Innis M A, Gelfand D H, Sninsky J J and White T J (Eds). Academic Press Inc, New York.

Verkley G J, Dukik K, Renfurm R, Göker M and Stielow J B. 2014. Novel genera and species of coniothyrium-like fungi in Montagnulaceae (Ascomycota). *Persoonia* **32**: 25–51.