First report of *Cercospora dianellicola* as an endophyte of *Paspalum notatum* (Poaceae)

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Abstract

The aim of this work was to study the fungal diversity in *Paspalum notatum*, in order to determine the potential biological control of disease-causing pathogens and their effects on growth promotion. Two isolates of *Cercospora dianellicola* were identified from asymptomatic *P. notatum* leaves, as an endophytic species. The possible benefits for the host plant remain to be analyzed.

Keywords *Cercospora* · *Paspalum* · Endophytes · Argentina

Paspalum is one of the best represented genera in South America rangelands (Acuña et al. 2019). *Paspalum notatum* (bahiagrass) stands out among the species of the genera because of its dominances in native grasslands in southern Brazil, Paraguay, and northeastern Argentina and for being cultivated as forage, turf, and a soil stabilizer around the world (Blount and Acuña 2009).

Fungal endophytes form symbiotic relationships with *Paspalum* species (García Lemos et al. 2017), which are important in the structure, function, and health of plant communities. The meaning of endophyte includes all organisms that live asymptotically within living plant aerial tissues, at least some period of their life cycle (Saikkonen et al. 1998). These organisms can comprise both saprophytic fungi and latent pathogens (Petrini et al. 1995).

Due to the interaction with endophytic fungi, physiological changes occur within plants. Increases in the photosynthetic rate, the absorption of phosphates, saline stress

tolerance, growth and disease resistance are among the reported benefits (Patle et al. 2018). Based on the promising results reported in fungal endophytes, it was decided to study the fungal diversity in *P. notatum*, to analyze the potential biological control of disease-causing pathogens and their effects on growth promotion.

Plant samples were collected from rangelands exposed to continuous grazing in Riachuelo, Corrientes, Argentina (S27°38'15"; W58°44'48"). Riachuelo has humid subtropical weather with an average annual rainfall of 1,300 mm and average annual temperature of 22 °C (Carnevali 1994).

A total of 84 plants were used for the isolation of endophytes, following the protocol reported by Depetris et al. (2020). Briefly, a triple-sterilization process was carried out by submerging three pieces of leaves and three pieces of stems (3–5 cm) from each plant in ethanol and sodium hypochlorite to eliminate surface colonizers. This process consisted of: (a) ethanol (90% v/v) for 1 min; (b) sodium hypochlorite [chlorine 25 g/l] (50% m/v) for 9 min; (c) ethanol (70% v/v) for 1 min; and (d) three rinses with sterilized distilled water and finally, dried on sterile filter paper. Then, six smaller pieces of 3–5 mm from larger sterilized parts of leaves and stems for each of the 84 plants described above, were placed on the surface of PDA (potato dextrose agar) in a Petri dish (90 × 15 mm) and were incubated in the dark for 30 days at 28 °C. Single hyphal tips were taken from the original cultures by cutting 5 × 5 mm pieces of agar from the edges of the colonies to obtain pure cultures. Once all pure cultures of each isolate were obtained, information of colony features were recorded. The mycelium from each isolated fungi were placed

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on microscope slides using a drop of Cotton Blue suspended in water, covered with a coverslip and observed microscopically. Sporulating fungi were tentatively identified using key morphological features, with the aid of data in Woudenberg et al. (2015), Manamgoda et al. (2015) and other publications.

To obtain molecular identification, DNA was extracted from dry mycelium (60–75 mg) from isolated pure cultures grown in sterile liquid medium (5 g of yeast extract, 5 g of peptone, 20 g of sucrose and distilled water to reach 1 L) in 15 mL Falcon tubes. The tubes were placed on an orbital shaker at 28 °C for 10–15 days after which the tubes were centrifuged, the supernatant discarded, and the pellet mycelium was oven-dried without a lid, for approximately 30 min. The dry mycelium was ground in a mortar with liquid nitrogen and mycelia were disaggregated in two consecutive incubations, the samples were treated with RNAases, and DNA concentrations were checked in agarose gels. In summary, specific primers (ITS1 and ITS4) were used to amplify sequences of the ITS region (ITS1-5.8S-ITS2),

using as a template 0.5–2 µg/ml genomic DNA. Agarose gels (1% m/v in 0.5X TBE) stained with SYBR SAFE (Invitrogen) were used to determine amplified bands size by establishing a molecular weight marker (100 bp Quick-load; New England Biolabs). These were sequenced by Macrogen Inc. (Korea) and analyzed with the Sequencher software, version 4.1.4 (demo). The length of the sequences ranged between 500 and 600 pb, as expected for the combination of primers (DDBJ accession numbers LC605628–LC605640). The ITS sequences of all the isolates were compared with other published sequences using the National Center for Biotechnology Information (NCBI; <http://www.ncbi.nlm.nih.gov>). Afterwards, the software Phylogeny was used to analyze all fungal sequences mentioned above (Dereeper et al. 2008). A dendrogram was obtained which allowed not only grouping by closeness of sequences, but also helped to classify isolates at genus or species level.

Forty-four isolates were obtained from the 84 plants and were identified as being species of *Alternaria*, *Aspergillus*,

Fig. 1 Phylogenetic analysis. Tree obtained from the alignment of ITS sequences (ITS1–5.8S–ITS2) of 13 unpublished isolates of endophytic fungi collected from plants of *P. notatum* (identified in

bold, with DDBJ accession number in brackets) and 20 sequences of the NCBI website (*Cercospora dianellicola* written in blue)

Fig. 2 *Cercospora dianthellicola* isolated from *P. notatum* grown on PDA medium showing its colony morphology (A front and B reverse)

Bipolaris, *Cercospora*, *Curvularia*, *Penicillium*, *Trichoderma* or were unidentified. Two isolates of a *Cercospora*-like species were identified from the molecular study. The ITS1 and ITS4 sequences of these two isolates [the two sequences of *Cercospora* isolates L and G were deposited in the DNA Data Bank of Japan (DDBJ), at the National Institute of Genetics (NIG) in the Shizuoka prefecture of Japan, with accession numbers LC605636 and LC605637, respectively] showed 99.4% and 99.8% similarity to the Type isolate (CPC 32,597) of *Cercospora dianthellicola* (Fig. 1).

Cercospora dianthellicola developed colonies with aerial mycelium, lobate margins, dark grey olivaceous surface and iron-grey in reverse (Fig. 2), and which were 40 ± 5 mm diameter after 1 month's incubation at 25 °C on PDA medium. Conidiophores and conidia were not observed. *C. dianthellicola* was originally described from a species of *Dianella* (F. iridiaceae) in Australia (Crous et al. 2017).

Several *Cercospora* species have been reported to cause leaf spot on *Paspalum* spp. (Chupp 1954; Braun et al. 2015), but none have been demonstrated to be endophytic in *Paspalum* plants. However, species of *Cercospora* have been reported as endophytes of a range of non-grass hosts, including *Glycine max*, *Phaseolus vulgaris*, *Pinus ponderosa*, *Coffea arabica*, *Gossypium hirsutum*, among others (Rashmi et al. 2019).

In conclusion, the results presented here provide evidence that *C. dianthellicola* is an endophyte in plants of *Paspalum notatum* in the rangelands of Riachuelo, Argentina. The possible benefits for the host plant remain unknown. Since there are reports that endophytes increase forage yield and its nutritive value (Santamaria et al. 2017), this endophyte could be affecting the performance of *P. notatum*. Further research is needed to determine the role that *C. dianthellicola* plays in the ecology of bahiagrass.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

References

- Acuña CA, Martínez EJ, Zilli AL, Brugnoli EA, Espinoza F, Marcón F, Urbani MH, Quarín CL (2019) Reproductive systems in *Paspalum*: relevance for germplasm collection and conservation, breeding techniques, and adoption of released cultivars. *Front Plant Sci* 10:1377. <https://doi.org/10.3389/fpls.2019.01377>
- Blount AR, Acuña CA (2009) Bahiagrass. In: Singh RJ (ed.) Genetic resources, chromosome engineering, and crop improvement series, Forage crops, vol. 5, Boca Raton, FL, CRC Press, pp 81–101. <https://doi.org/10.1201/9781420047400.ch4>
- Braun U, Crous PW, Nakashima C (2015) *Cercosporoid* fungi (*Mycosphaerellaceae*) 3. Species on monocots (*Poaceae*, true grasses). *IMA Fungus* 6:25–98
- Carnevali R (1994) Fitogeografía de la provincia de Corrientes. Instituto Nacional de Tecnología Agropecuaria, Corrientes
- Chupp C (1954) A monograph of the fungus genus *Cercospora*. Ithaca, New York
- Crous PW, Groenewald JZ, Wingfield MJ, Summerell BA, Carnegie AJ (2017) Fungal Planet description sheets. <http://www.fungalplanet.org/content/pdf-files/FungalPlanet705.pdf>
- Depetris MB, Acuña CA, Gutierrez S, Marcón F, Felitti SA (2020) Fungal endophyte diversity in *Paspalum* and effects on plant growth. *Grass Forage Sci* 75:316–325. <https://doi.org/10.1111/gfs.12494>
- Dereeper A, Guignon V, Blanc G, Audic S, Buffet S, Chevenet F, et al. (2008) Phylogeny.fr: Robust phylogenetic analysis for the non-specialist. *Nucleic Acids Res* 36:465–469. <https://doi.org/10.1093/nar/gkn180>
- García Lemos A, Gil Cardeza ML, Gómez EV, Felitti SA (2017) Fungal endophytes in species of *Paspalum* and *Bromus*, occurrence and *in vitro* antagonistic activity assessment. *Res & Rev: Res J Biol* 5:15–25
- Manamgoda DSD, Rossman AY, Castlebury LA, Chukeatirote K, Hyde D (2015) A taxonomic and phylogenetic re-appraisal of the genus *Curvularia* (Pleosporaceae): human and plant pathogen. *Phytotaxa* 212:173–198
- Patle PN, Navnag NP, Ramteke PR (2018) Endophytes in plant system: Roles in growth promotion, mechanism and their potentiality in achieving agriculture sustainability. *Int J Chem Stud* 6:270–274
- Petrini O, Petrini L, Rodrigues K (1995) Xylariaceous endophytes: An Exercise in Biodiversity. *Fitopatol Bras* 20:531–539
- Rashmi M, Kushveer JS, Sarma VV (2019) A worldwide list of endophytic fungi with notes on ecology and diversity. *Mycosphere* 10:798–1079. <https://doi.org/10.5943/mycosphere/10/1/19>
- Saikkonen K, Faeth S, Helander M, Sullivan T (1998) Fungal endophytes: A continuum of interactions with host plants. *Annu Rev Ecol Evol Syst* 29:319–343. <https://doi.org/10.1146/annurev.ecolsys.29.1.319>
- Santamaria O, Lledó S, Rodrigo S et al (2017) Effect of Fungal Endophytes on Biomass Yield, Nutritive Value and Accumulation of Minerals in *Ornithopus compressus*. *Microb Ecol* 74:841–852. <https://doi.org/10.1007/s00248-017-1001-3>
- Woudenberg JH, Seidl MF, Groenewald JZ, de Vries M, Stielow JB, Thomma BP, Crous PW (2015) Alternaria section Alternaria: species, formae speciales or pathotypes? *Stud Mycol* 82:1–21