COMUNICAÇÕES

Alternaria alternata causing foliar blight on *Chrysanthemum morifolium* in Corrientes, Argentina

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In Corrientes, province of Argentina, *Chrysanthemum morifolium* Ramat (Asteraceae), is the second most important crop of cut flowers, following gladiolus. Chrysanthemum is a type of floral plants frequently affected by several diseases such as white rust (*Puccinia horiana*), withering (*Fusarium* sp. and *Verticillium* sp.), spots and foliar blight caused by *Alternaria* spp. and *Septoria* sp. (1). In Corrientes Province, this disease produces losses of 60% plants in greenhouses. Thus, new observations and studies have been conducted to get to know the species of *Alternaria* that is involved in the disease affecting chrysanthemum in Corrientes. Samples showing symptoms of foliar blight were collected from crops in Concepción and Corrientes Cities at different times of the year during the growing period (Fig. 1A).

The diseased plant material was washed in running tap water, surface disinfected with 10% sodium hypochlorite solution, and rinsed with distilled water. After surface disinfection, plant tissues were cut into small fragments, which were placed in plates containing potato-glucose-agar (PGA-2% w/v, pH 6.6-7) for the development of fungal colonies under laboratory conditions of 12h light-12h darkness photoperiod, at 25°C (3). After purification of strains through monosporic cultivation, the fungal colonies were preserved in test tubes with sloped PGA. The fungal isolate of interest, M5, was deposited in the culture collection of the Department of Plant Protection (National University of Northeast) and National University of Misiones, receiving the accession number LBM 212. The morphological characteristics were established considering the study of pure strains obtained from one spore of each isolated strain cultivated in PGA (2%, pH 6.6 -7). Cultural characteristics were recorded from the isolate cultivated in PGA, which was developed under conditions of 12h light-12h darkness, at 25° C. Furthermore, macroscopic characteristics of each developed fungal colony were described based on the observation of the following parameters: growth rate, aspect and top and bottom color of the colony. Microscopic features like characteristics of the mycelium, conidiophores and conidia were also analyzed for morphological identification under an Enosa optical microscope (400x), using fresh material from diseased leaves, stems and flowers mounted on sterile water.

Mycelia for DNA extraction were grown in liquid cultures at 28 ±1 °C in malt extract broth for molecular identification. Amplifications were confirmed by standard gel electrophoresis, using 2% w/v agarose gels (InBio, Argentina) in 0.5X TBE Buffer, and staining with Gel Red Solution (Biotium, 10000X). After amplification, the amplicons were

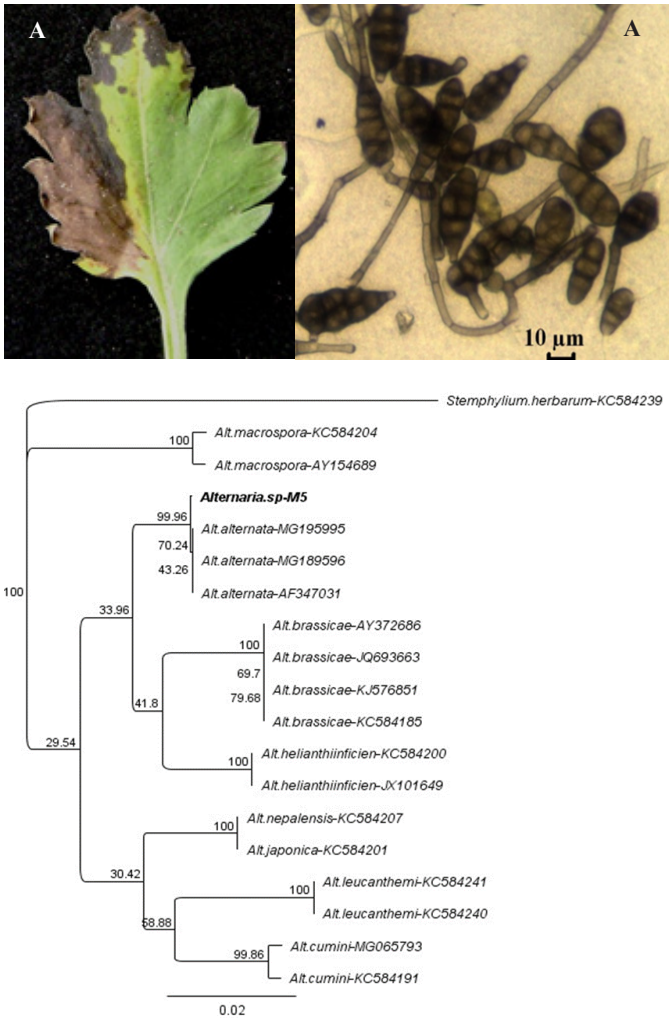

Figura 1. Symptoms of foliar blight on a fungal-inoculated chrysanthemum leaf (A); Conidial structures of *Alternaria alternata* on chrysanthemum (B); Neighbor-Joining distance tree analysis showing the phylogenetic relationships of *Alternaria* genus. A neighbor-joining tree was performed using ITS rDNA sequences. The sequence of our *A. alternata* strain is indicated in bold letters. Numbers on branches correspond to bootstrap values obtained with 5,000 replicates (C).

sent to Macrogen Korea for sequencing in both directions to corroborate the PCR product.

The ITS rDNA sequence generated in this study was deposited in GenBank (National Center for Biotechnology Information) under the accession number MG282916. The ITS sequence of the fungal strain was compared with sequences in Fungal Barcoding for molecular identification.

To analyze the isolated *Alternaria* strain, 18 accessions of the ITS rDNA sequences were selected and retrieved from the GenBank database representing species within the *Alternaria* genus. The obtained nucleotide sequence consists of approximately 600 bp which correspond to the ITS rDNA. A sequence of *Stemphylium herbarum* (KC584239) was used as outgroup. DNA sequences were aligned and a Neighbor-joining genetic distance tree was constructed with Geneious software, version 8.1.8 (3, 4). Support for specific clades represented in the tree was estimated by Bootstrap analysis of 5,000 replicates. Phylogenetic data were submitted to TreeBase under the submission number S21750 (Fig. 1C).

Pathogenicity tests consisted in applying two treatments (with and without injuries) of 4 replicates, using the method of loose-leaves in sterile plastic boxes and damp paper as substrate. The incubation process was conducted under controlled laboratory conditions in lighting chambers.

The mycelium of *Alternaria* strain No. 5 grew immersed in the substrate or partially covered; the hyphae were colorless when young but turned brown as they grew. The conidiophores were straight or flexuous, septate, variable in length, between 22.4 - 72.8 µm, and brown. In general, conidia were obpiriformis, brown, longitudinal and cross-cutting (Fig. 1B). Each conidium had a short slightly clearer and swollen beak, with a black scar. The conidia exhibited remarkable constrictions in the septa and grew in isolates forming medium-sized chains over the conidiophore. Its size varied. The length of the conidial body was between 8.4 µm and 19.6 µm, meanwhile the length of the beak varied between 0 and 6.67 µm. The width of the conidium was 4.2 - 8.4 µm. The number of transversal septs was between 1 and 7, with fewer longitudinal and oblique septs. These features of the fungus coincided with the features of *A. alternata* species described by Ellis (2).

Alternaria isolate M5 sequence was analyzed and compared to the fungal sequences available in molecular databases. The analyzed sequence blasted with sequences of different isolates of *Alternaria alternata* in the curated molecular database of Fungal Barcoding, showing 100% similarity.

According to morphological exams, the fungi causing blight symptoms in chrysanthemum plants resulted similar to *A. alternata*, which was confirmed with molecular studies.

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