NOTAS CIENTÍFICAS

Characterization of *Alternaria* isolates causing leaf spots in radish in Brazil

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Data de chegada: 07/02/2020. Aceito para publicação em: 14/10/2020

10.1590/0100-5405/233817

ABSTRACT

Cabral, C.S.; Barboza, E.A.; Lopes, L.H.R.; Rossato, M.; Borges, R.C.F.; Reis, A. Characterization of *Alternaria* isolates causing leaf spots in radish in Brazil. ***Summa Phytopathologica,*** v.46, n.4, p.340-341, 2020.

Alternaria japonica Yoshii, an important cruciferous phytopathogenic fungus, has been identified in radish plants showing symptoms of necrotic spots with chlorotic halos. The samples were collected from the cities of Brasília-DF and Guaraciaba do Norte-CE, Brazil. The isolates are deposited in the collection of fungi and oomycetes of "Embrapa Hortaliças". Using the concept of morphological and phylogenetic species, two isolates were selected (EH-945 and EH-1379) for identification. Through the evaluation of morphological markers, the isolates were concluded to be similar to *A. japonica*. Based on the phylogenetic analysis, the isolates grouped with *A. japonica* reference isolates ATCC 13618 and CBS 118390. To complete Koch's postulates, radish, arugula, mustard and turnip plants were inoculated. All species showed symptoms similar to those originally reported in the field (except for non-inoculated controls) seven to 12 days after inoculation. The isolates obtained from symptomatic plants showed morphological characteristics identical to those of the pathogen. This is the first report of radish as a host of *A. japonica* in Brazil.

Keywords: Etiology, morphology, phylogeny, *Raphanus sativus*

RESUMO

Cabral, C.S.; Barboza, E.A.; Lopes, L.H.R.; Rossato, M.; Borges, R.C.F.; Reis, A. Caracterização de isolados de *Alternaria* causando manchas foliares em rabanete no Brasil. ***Summa Phytopathologica,*** v.46, n.4, p.340-341, 2020.

Alternaria japônica Yoshii, um importante fungo fitopatogênico das crucíferas, foi identificado em plantas de rabanete apresentando sintomas de manchas necróticas com halos cloróticos. As amostras foram coletadas nos municípios de Brasília-DF e Guaraciaba do Norte-CE, Brasil. Os isolados estão depositados na coleção de fungos e oomicetos da Embrapa Hortaliças. Com o emprego do conceito de espécie morfológica e filogenética, dois isolados foram selecionados (EH-945 e EH-1379) para identificação. Através da avaliação dos marcadores morfológicos, concluiu-se que os isolados estudados são semelhantes a *A. japonica.* Baseado na análise filogenética, os isolados agruparam com os isolados de referências ATCC 13618 e CBS 118390 de *A. japonica*. Visando completar os postulados de Koch foram inoculadas plantas de rabanete, rúcula, mostarda e nabo. Todas as espécies apresentaram sintomas similares aos relatados originalmente em campo (com exceção dos controles não inoculados), sete a 12 dias após a inoculação. Os isolados obtidos das plantas sintomáticas apresentaram características morfológicas idênticas às do patógeno. Esse é o primeiro relato de rabanete como hospedeira de *A. japonica* no Brasil.

Palavras-chave: Etiologia, filogenia, morfologia**,** *Raphanus sativus*

Radish (*Raphanus sativus* L.) is a root vegetable of the family Cruciferae (*Brassicaceae*) which can be infected by *Alternaria* spp. (causal agent of leaf spots). At least three *Alternaria* species can cause leaf spots to plant species of the Brassicaceae family (6). The species *A. brassicae* (Berk.) Sacc. 1880 and *A. brassicicola* (Schwein.) Wiltshire 1947 are highly spread among almost all Brassicaceae producing regions in Brazil, attacking a large number of leaf vegetables (4). The species *A. japonica* Yoshii 1941 (Sin. *A. raphani* J.W. Groves & Skolko 1944) has more restricted host range and geographic distribution (3).

Severe leaf spot symptoms such as black necrotic lesions surrounded by chlorotic areas (Figure 1B) were observed at two commercial radish fields in 2004 and 2009 in Brasília-DF and Guaraciaba do Norte-CE, respectively. Disease incidence was 100% in Brasília and approximately 75% in Guaraciaba do Norte. Leaf samples were collected from both fields and taken to the Plant Pathology Laboratory of "Embrapa Hortaliças". Fragments of these leaves were cut and disinfested in 70% ethyl alcohol and 0.5% sodium hypochlorite. These fragments were maintained in moist chambers for two days, when monosporic isolates were obtained in water-agar media.

Two fungal isolates were obtained from each field. For morphological characterization and production of asexual structures, the isolates were cultivated on potato carrot agar (PCA) and 10% V8 media (pH 6.4), at 22ºC and 8h photoperiod, for seven days (5). Colonies on PCA were initially pale to olive-grey, becoming darker with time. Morphological analyses of those isolates revealed olive-brown conidiophores, mostly unbranched, highly variable in length, 4-8 µm diameter, and slightly enlarged at apical conidiogenous cell. Conidia (n=30) were ovoid (37.64 to 59.58 x 19.33 to 26.61 µm) with only 2-3

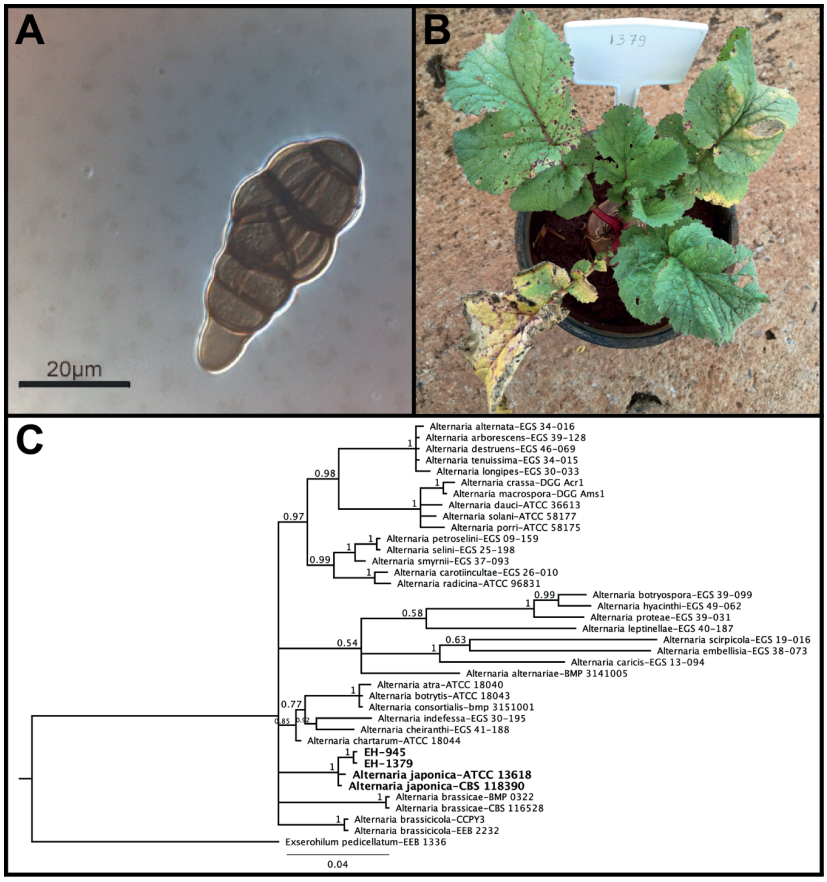

Figure 1. A – Ellipsoid conidium with transverse septa and longisepta. **B** – Alternaria leaf spot symptoms in radish. **C** - Bayesian inference phylogenetic tree (model GTR+G+I, 4 million generations) with GAPDH region of the two isolates from the present study (EH-945 and EH-1379) along other *Alternaria* accessions from GenBank.

transverse septa plus 1-2 longisepta in 1-3 transverse segments and were borne singly or in short chains of 2-3 conidia (Figure 1A). These characteristics are similar to those described for *Alternaria japonica* (5).

To confirm the etiological agent of the disease, total genomic DNA of two isolates, 'EH-945' and 'EH-1379', was extracted and used in PCR assays with primers GPD-1 and GPD-2 (1) targeting the glyceraldehyde-3-dehydrogenase gene (GAPDH). Amplicons were then sequenced and deposited in GenBank as MK510949 and MK510950.

Nucleotide BLAST showed that both sequences ('EH-945' and 'EH-1379') had 99.1% identity with isolate RGW9 of *A. japonica*. Bayesian inference analysis (GTR+G+I) using several *Alternaria* species clustered 'EH-945' and 'EH-1379' with *A. japonica* isolates ATCC 13618 and CBS 118390 with the value of "1.00" of posterior probability (Figure 1C). Pathogenicity assays were performed under greenhouse conditions by inoculating the two isolates in seedlings of radish, arugula (*Eruca sativa* Mill.), wild radish (*Raphanus raphanistrum* L.) and mustard [*Brassica juncea* (L.) Czem.] with 6 ml of a suspension containing 1 x 10^4 conidia/ml. Ten plants of each species were inoculated and kept for three days in moist chambers made of plastic bags. All inoculated plants presented the same field symptoms of leaf spots seven days after inoculation. Control plants sprayed with sterile water remained symptomless. Re-isolation of the pathogen from symptomatic plants was performed and showed colonies with the same previously seen characteristics, fulfilling Koch's postulates.

The fungal isolates were pathogenic to all tested plant species but were more aggressive in radish. Both isolates were reisolated from all inoculated plants and their morphological characteristics were the same as those of the original isolates. The species *A. japonica* (*A. raphani*) has been reported as the causal agent of leaf spot for other hosts in Brazil (3) and for *R. sativus* in different countries (2). However, to the best of our knowledge, this is the first report of radish as a host of *A. japonica* in Brazil.

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