Alternaria dauci causes leaf spots and leaf blight of coriander (*Coriandrum sativum*) in Brazil

Paloma S. Mansur¹ · André L. Silva¹ · Sara S. Salcedo¹ · Robert W. Barreto¹ · Adans A. Colmán^{1,2}

Received: 18 November 2019 / Accepted: 8 October 2020 / Published online: 19 October 2020
© Australasian Plant Pathology Society Inc. 2020

Abstract

Alternaria dauci is fully confirmed to cause leaf spots and leaf blight of coriander (*Coriandrum sativum*) in Brazil based on observations made of blighted plants at a demonstration plot (Infectarium) at Viçosa (state of Minas Gerais) Brazil. Identification was supported by a combination of morphological, cultural and molecular features. A representative herbarium sample and an isolate were deposited in public collections as well as DNA sequences. Pathogenicity tests were conducted and Koch's postulates were fulfilled.

Keywords Etiology · Phylogeny · Section *Porri* · Pathogenicity · Pleosporaceae

Coriander (*Coriandrum sativum*), also known as cilantro (coentro in Brazil) is an annual herb of the Apiaceae. It is widely cultivated and used as a condiment herb worldwide (Simpson and Connor-Ogorzaly 2001) and also in Brazil. Several fungal pathogens have been reported as causing leaf spots on coriander in Brazil (Farr and Rossman 2019) and *Alternaria* leaf spot is considered an important disease of coriander in Brazil (Reis and Lopes 2016).

In July 2018, all plants growing in a demonstration plot in the Infectarium – the plant disease garden - of the Departamento de Fitopatologia of the Universidade

Federal de Viçosa (municipality of Viçosa, state of Minas Gerais, Brazil) were found to be attacked by a disease leading to severe leaf blight symptoms. Firstly small round to oval, 3–5 mm diam., leaf spot appeared which quickly coalesced and turned into necrotic blight of entire leaves. Samples were collected and examined under a dissecting microscope and a dematiaceous fungus was found to be regularly associated with the necrotic tissues. Under a dissecting microscope (Olympus SZX7) conidia were taken from infected leaves with a sterile fine pointed needle and transferred onto previously marked positions of V8 juice-agar plates. Each position of those plates was then examined, under the high power of the dissecting microscope, to select positions where single conidia were deposited. Those points of the plates were further marked, at the underside of the plates, indicating that colonies from these positions represented single-spore isolates. After 1 week of growth in the incubator, at 25 °C, a fragment of the margin of one selected single spore colony was taken and transferred to a tube containing potato carrot-agar. This representative culture was deposited in the culture collection of the Universidade Federal de Viçosa (Acc No COAD 2594) and used in this study. Additionally, a representative specimen of diseased coriander was dried in a plant press and deposited in the local herbarium (Acc. No VIC 47142).

Slides were prepared by scraping fungal structures from sporulating colonies on leaves and mounting these on a drop

✉ Adans A. Colmán
adan-colman@hotmail.com

Paloma S. Mansur
paloma.mansur@ufv.br

André L. Silva
als9528@gmail.com

Sara S. Salcedo
taphrina10@gmail.com

Robert W. Barreto
rbarreto@ufv.br

¹ Departamento de Fitopatologia, Universidade Federal de Viçosa, Viçosa, Minas Gerais 36570-900, Brazil

² Present address: Facultad de Ciencias Agrarias, Universidad Nacional de Asunción, San Lorenzo, Paraguay

of lactophenol. Observations were made under a microscope (Olympus BX 53) equipped with differential interference contrast (DIC) illumination and connected to an Olympus Q-color 5™ camera. The fungus on coriander had the following morphology: conidiophores forming loose groups, subcylindrical, straight to somewhat sinuose, $27.5\text{--}75 \times 5\text{--}10\text{ }\mu\text{m}$, 1–4 septate, pale brown to brown, smooth; conidiogenous cells subcylindrical, geniculate, $(7.5)\text{ }15\text{--}27.5\text{ } (37.5) \times 5\text{--}10\text{ }\mu\text{m}$, conidiogenous loci terminal to intercalary $2.5\text{--}7.5\text{ }\mu\text{m}$ diam.; conidia solitary, $(125)\text{ }200\text{--}300\text{ } (340) \times 17.5\text{--}22\text{ }\mu\text{m}$, 1–8 longitudinally and 8–15 transversally septae, beak up to $280\text{ }\mu\text{m}$ long, pale brown, walls somewhat verruculose (Fig. 1).

DNA was extracted from mycelium of COAD 2594 grown in 20 ml of potato dextrose broth at $23 \pm 2\text{ }^{\circ}\text{C}$ for 4 days. Extraction was performed with a Wizard Genomic DNA Purification Kit (Promega) according to the manufacturer's recommendations. The glyceraldehyde 3-phosphate dehydrogenase (GAPDH) region was amplified with the primers gpd1 and gpd2 (Berbee et al. 1999), the RPB2 region with RPB2-5F2 (Sung et al. 2007) and fRPB2-7cR (Liu et al. 1999) and the Alt a1 region with the primers Alt-for and Alt-rev (Hong et al. 2005). PCR was performed following the procedure described in Colmán et al. (2018) and phylogenetic analyses were performed as described in Woudenberg et al. (2013). Bayesian inference analyses were performed with the CIPRES web portal using MrBayes v. 3.2.6. (Miller et al. 2015). The jmodeltest (Darriba et al. 2012) was used for estimation of sequence evolution. Based on the phylogenetic analyses results combined GAPDH, Alt 1 and RPB2 gene regions, our isolate COAD 2594 was identified as belonging to *Alternaria dauci* (Fig. 2). Sequences obtained in this work were deposited in GenBank (Table 1).

To confirm the pathogenicity of COAD 2594, 4 month old healthy coriander plants were inoculated. Additionally, groups of four pots (one healthy plant per pot) containing either carrot, celery or parsley were also inoculated with COAD 2594. A conidial suspension was prepared by flooding sporulating 7-day-old cultures formed on V8 juice-agar plates with sterile distilled water (SDA) and scraping their surface with a rubber spatula. A haemocytometer was used to calculate the concentration of spores/ml which was then adjusted to 10^6 conidia/ml. Plants were sprayed until runoff and covered with a plastic bag for 48 h to keep humidity levels high. One healthy plant of each species treated similarly but sprayed with SDA only served as controls. Typical symptoms of disease were observed 2–4 days after inoculation. Control plants remained healthy. *Alternaria*

Fig. 1 *Alternaria dauci* on coriander (*Coriandrum sativum*). **a** Leaf spot and some blight symptoms on coriander; **b** Group of conidiophores of *A. dauci*; **c** Conidia with long septate beaks, **d** Colony of *A. dauci* on V8 juice-agar; **e–f** Pathogenicity test on coriander plants, control (**e**) and inoculated plants (**f**) 4 days after inoculation. Scale bars $20\text{ }\mu\text{m}$

dauci sporulated abundantly on necrotic tissues and was reisolated on V8 juice-agar plates producing sporulating cultures. The morphology of the fungus reisolated from necrotic tissues of inoculated plants was examined under the microscope and found to have a morphology identical to COAD 2594 fulfilling Koch's postulates.

Several species of *Alternaria* have been reported on *C. sativum* (Farr and Rossman 2019). Of these (*A. poonensis*) was originally described by Raghunath

Table 1 GenBank accession number of sequences obtained in this study and other *Alternaria* spp. used for phylogenetic analysis

Species Name	Host	Strain number	Country	GenBank accession number		
				Alt 1	GPDH	RPB2
<i>Alternaria anagallidis</i>	<i>Anagallis arvensis</i>	CBS 117128	New Zealand	KJ718628	KJ717961	KJ718282
<i>Alternaria anagallidis</i>	<i>Anagallis arvensis</i>	CBS 117129	New Zealand	KJ718629	KJ717962	KJ718283
<i>Alternaria argyroxiphii</i>	<i>Argyroxiphium</i> sp	CBS 117122	USA	KJ71863	JQ646350	KJ718286
<i>Alternaria argyroxiphii</i>	<i>Ipomoea batatas</i>	PPRI 11848	South Africa	KJ718633	KJ717965	KJ718287
<i>Alternaria argyroxiphii</i>	<i>Ipomoea batatas</i>	PPRI 11971	South Africa	KJ718634	KJ717966	KJ718288
<i>Alternaria bataticola</i>	<i>Ipomoea batatas</i>	CBS 531.63	Japan	JQ646433	JQ646349	KJ718291
<i>Alternaria bataticola</i>	<i>Ipomoea batatas</i>	CBS 532.63	Japan	KJ718637	KJ717969	KJ718292
<i>Alternaria bataticola</i>	<i>Ipomoea batatas</i>	CBS 117095	Australia	KJ718638	KJ717970	KJ718293
<i>Alternaria bataticola</i>	<i>Ipomoea batatas</i>	CBS 117096	Australia	KJ718639	KJ717971	KJ718294
<i>Alternaria dauci</i>	<i>Daucus carota</i>	CBS 111.38	Italy	KJ718673	KJ718005	KJ718331
<i>Alternaria dauci</i>	<i>Daucus carota</i>	CBS 345.79	New Zealand	KJ718675	KJ718007	KJ718333
<i>A. dauci</i> (syn. <i>A. poonensis</i>)	<i>Coriandrum sativum</i>	CBS 117.100	Puerto Rico	KJ718680	JQ646348	KJ718338
<i>Alternaria dauci</i>	<i>Coriandrum sativum</i>	USFL-3	USA	MF595071	MF595072	–
<i>Alternaria dauci</i>	<i>Coriandrum sativum</i>	NB625	Algeria	–	MK513412	MK513423
<i>Alternaria dauci</i>	<i>Coriandrum sativum</i>	NB622	Algeria	–	MK513409	MK513420
<i>Alternaria dauci</i>	<i>Coriandrum sativum</i>	COAD 2594	Brazil	MN433697	MT050451	MN433698
<i>Alternaria echinaceae</i>	<i>Echinaceae</i> sp.	CBS 116117	New Zealand	KJ718684	KJ718015	KJ718343
<i>Alternaria echinaceae</i>	<i>Echinaceae</i> sp.	CBS116118	New Zealand	KJ718685	KJ718016	KJ718344
<i>Alternaria euphorbiicola</i>	<i>Euphorbia hyssopifolia</i>	CBS 133874	USA	KJ718019	KJ718687	KJ718347
<i>Alternaria euphorbiicola</i>	<i>Euphorbia pulcherrima</i>	CBS 19886	USA	KJ718017	KJ718686	KJ718345
<i>Alternaria ipomeae</i>	<i>Ipomoea batatas</i>	CBS 219.79	Ethiopia	KJ718689	KJ718020	KJ718348
<i>Alternaria ipomeae</i>	<i>Ipomoea batatas</i>	PPRI 8988	South Africa	KJ718690	KJ718021	KJ718349
<i>Alternaria neoipomeae</i>	<i>Ipomoea batatas</i>	PPRI 8990	South Africa	KJ718706	KJ718035	KJ718370
<i>Alternaria neoipomeae</i>	<i>Ipomoea batatas</i>	PPRI 13903	South Africa	KJ718709	KJ718038	KJ718373
<i>Alternaria tagetica</i>	<i>Tagetes</i> sp.	CBS 297.79	UK	KJ718759	KJ718080	KJ718428
<i>Alternaria tagetica</i>	<i>Tagetes</i> sp.	CBS 298.79	UK	KJ718760	KJ718081	KJ718429
<i>Alternaria tagetica</i>	<i>Tagetes erecta</i>	CBS 479.81	UK	KJ718761	KC584143	KC584434
<i>Alternaria tropica</i>	<i>Passiflora edulis</i>	CBS 631.93	USA	KJ718768	KJ718088	KJ718436
<i>Alternaria tropica</i>	<i>Passiflora edulis</i>	CBS 117216	USA	KJ718769	KJ718089	KJ718437

(1963) based on a sample of diseased coriander from India. This was based on morphological features and supposed host-specificity. Morphology of COAD 2594 was found to be rather similar to that described by Raghunath (1963) for *A. poonensis*, including for the absence of bifurcated beaks, thought to be typical for *A. dauci* but not seen in COAD 2594 (either from *in planta* or in vitro materials). Nevertheless, based on their molecular phylogenetic analysis Woudenberg et al. (2014), placed *A. poonensis* as a synonym of *A. dauci*. Results of our phylogenetic analysis of COAD 2594 were in agreement with Woudenberg et al. (2014) (Fig. 2). COAD 2594 grouped with the type strain of *A. dauci* (CBS 111.38) and other *A. dauci* strains isolated from coriander in the United

States US-FL-1 (Poudel and Zhang 2018) and Algeria NB622 (Bessadat et al. 2019). *Alternaria dauci* is now recognized to be a polyphagous pathogen infecting several plants in the Apiaceae. COAD 2594 was also proven to be capable of infecting carrots, parsley and celery in our inoculation studies, further confirming the polyphagous status (within the Apiaceae) of *A. dauci*. In Brazil this species has already been reported in association with coriander seeds and plants (Reis et al. 2006). Nevertheless these reports were never supported by molecular data and no voucher specimens were deposited in herbaria or culture collections. Here the identity of *Alternaria dauci* as the ethiological agent of leaf spots and blight of coriander in Brazil is fully confirmed for the first time.

Fig. 2 Multilocus phylogenetic tree of *Alternaria* species inferred from Bayesian analysis based on combined Alt a 1, GAPDH and RPB2 sequences. Bayesian posterior probabilities above 0.7 are indicated above the nodes. Isolate from Brazil are highlighted in bold. The tree was rooted with *Alternaria euphorbiicola* (CBS 133874 and CBS 198.86) isolates

References

- Berbee ML, Pirseyedi M, Hubbard S (1999) *Cochliobolus* phylogenetics and the origin of known, highly virulent pathogens, inferred from ITS and glyceraldehyde-3-phosphate dehydrogenase gene sequences. *Mycologia* 91:964–977
- Bessadat N, Hamon B, Bataillé-Simoneau N, Mabrouk K, Simoneau P (2019) First report of *Alternaria dauci* causing leaf blight of coriander (*Coriandrum sativum*) in Algeria. *Plant Dis* 103:2471. <https://doi.org/10.1094/PDIS-03-19-0582-PDN>
- Colmán A, Freitas L, Barreto RW (2018) First report of *Seimatosporium hypericinum* causing leaf spots on the ornamental shrub *Hypericum × inodorum* and in Brazil. *Aust Plant Dis Notes* 13:38
- Darriba D, Taboada GL, Doallo R, Posada D (2012) jModelTest 2: more models, new heuristics and parallel computing. *Nat Methods* 9:772
- Farr DF, Rossman AY (2019) Fungal database, U. S. National Fungus Collections, ARS, USDA. <https://nt.ars-grin.gov/fungalatabases/>. Accessed 11 June 2019
- Hong SG, Cramer RA, Lawrence CB, Pryor BM (2005) Alt a 1 allergen homologs from *Alternaria* and related taxa: analysis of phylogenetic content and secondary structure. *Fungal Genet Biol* 42:119–129
- Liu YJ, Whelen S, Hall BD (1999) Phylogenetic relationships among ascomycetes: evidence from an RNA polymerase II subunit. *Mol Biol Evol* 16:1799–1808
- Miller MA, Schwartz T, Pickett BE, He S, Klem EB, Scheuermann RH, Passarotti M, Kaufman S, O'Leary MA (2015) A RESTful API for access to phylogenetic tools via the CIPRES science gateway. *Evol Bioinforma* 11:43–48. <https://doi.org/10.4137/EBO.S21501>
- Poudel B, Zhang S (2018) First report of *Alternaria* leaf spot of cilantro (*Coriandrum sativum*) caused by *Alternaria dauci* in the United States. *Plant Dis*. 102: <https://doi.org/10.1094/PDIS-08-17-1297-PDN>
- Raghunath T (1963) A new species of *Alternaria* causing blight of *Coriandrum sativum* L. *Mycopathologia* 21:315–317
- Reis A, Satelis JF, Pereira RS, Nascimento WM (2006) Associação de *Alternaria dauci* e *A. alternata* com sementes de coentro e eficiência do tratamento químico. *Hortic Bras* 24:107–111
- Reis A, Lopes CA (2016) Doenças do coentro no Brasil. Brasília, DF: Embrapa (Embrapa Hortaliças) Circular Técnica, 157:1–6 <https://ainfo.cnptia.embrapa.br/digital/bitstream/item/157359/1/CT-157.pdf> Accessed 10 May 2019
- Simpson BB, Connor-Ogorzaly M (eds) (2001) *Economic botany: plants in our world*. McGraw-Hill, New York

- Sung GH, Sung JM, Hywel-Jones NL, Spatafora JW (2007) A multi-gene phylogeny of Clavicipitaceae (Ascomycota, fungi): identification of localized incongruence using a combinatorial bootstrap approach. *Mol Phylogenet Evol* 44:1204–1223
- Woudenberg JHC, Groenewald JZ, Binder M, Crous PW (2013) *Alternaria* redefined. *Stud Mycol* 75:171–212
- Woudenberg JHC, Truter M, Groenewald JZ, Crous PW (2014) Large-spored *Alternaria* pathogens in section Porri disentangled. *Stud Mycol* 79:1–49