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Species diversity, novel interactions and absence of well-supported host-guided phylogenetic groupings of Neotropical *Alternaria* isolates causing foliar lesions in Solanaceae.

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Summary

Aim: To report the characterization of 120 *Alternaria* isolates inducing early blight-like foliar lesions in nine species of five Solanaceae genera collected across all macro-geographical Brazilian regions.

Material and results: Phylogenetic relationships were assessed via analyses of the *Alternaria alternata* allergenic protein-coding, glyceraldehyde-3-phosphate dehydrogenase, and the calmodulin gene sequences. Most of the tomato isolates were placed into the *A. linariae* cluster, whereas most of the potato isolates were grouped with *A. grandis*. Novel host-pathogen interactions were also reported. Seventeen isolates were selected for morphometrical characterization and a subsample of 13 isolates was employed in pathogenicity assays on tomato, potato, eggplant, scarlet eggplant, *Capsicum annuum*, *Datura stramonium*, *Physalis angulata* and *Nicotiana tabacum*. Eleven isolates were able to induce foliar lesions in tomatoes but none in *C. annuum*. Potato was susceptible to a subgroup of isolates but displayed a subset of isolate-specific interactions. Morphological traits were in overall agreement with molecular and host range data.

Conclusion: *Alternaria linariae* and *A. grandis* were confirmed as the major causal agents of tomato and potato early blight, respectively. However other *Alternaria* species are also involved with early blight in solanaceous hosts in Brazil.

Significance and impact of the study: The diversity and host-specificity patterns of the *Alternaria* isolates from Solanaceae may have practical implications in establishing effective early blight genetic resistance and cultural management strategies especially for tomato and potato crops.

Key words: Early blight – Neotropical *Alternaria* – potato – *Solanum lycopersicum* – *Solanum tuberosum* – tomato.

Introduction

The Solanaceae is a hyper-diverse botanical family, which encompasses many genera and species of horticultural and industrial importance as well as many native, ornamental and weed species (Olmstead *et al.* 2008). *Solanum* is the largest genus within the family with approximately 1,700 species (Weese and Bohs 2007). The main crops within the Solanaceae family are potato

(*Solanum tuberosum* L.), tomato (*S. lycopersicum* L.), eggplant (*S. melongena* L.), scarlet eggplant (*S. aethiopicum* L. var. *gilo* Raddi), hot and sweet-pepper (species of the genus *Capsicum* L.), tamarillo (*S. betaceum* Cav.), tobacco (species of the genus *Nicotiana* L.), petunia (genus *Petunia* sensu Jussieu), and golden berry (*Physalis peruviana* L.) among other minor crops of regional importance. The most important solanaceous weeds are classified within the genera *Solanum*, *Physalis*, *Nicandra*, and *Datura*. In addition, there are many semi-domesticated, feral, and endemic species, especially within the genus *Solanum* subgenus *Leptostemonum*, which are known as ‘spiny solanums’ (Levin *et al.* 2006). This group contains the Neotropical species such as *S. lycocarpum* St.-Hill. (= lobeira or wolf-fruit) as well as other spiny *Solanum* species which are collectively named as ‘jurubebas’ and are also endemic to the Neotropical Latin America (Levin *et al.* 2006; Michereff-Filho *et al.* 2012).

Many fungal species of genus *Alternaria* Nees are plant pathogens that are dispersed under natural conditions by spores (conidia) carried by the wind and can be transmitted at long distances by contaminated seeds. The classical taxonomic approaches for species delimitation within the genus *Alternaria* were based upon conidial morphology (Simmons 2000, 2007), with an estimate number of 280 species occurring worldwide (Gannibal 2015). In this complex taxonomic scenario, additional analyses of DNA sequences of a collection of different genomic regions were required to refine the classification and phylogeny of *Alternaria* species (Pryor and Gilbertson 2000; Chou and Wu 2002; Pryor and Bigelow 2003; Hong *et al.* 2005; Runa *et al.* 2009; Lawrence *et al.* 2013, 2014; Woudenberg *et al.* 2013; Manamgoda *et al.* 2014). The pioneering studies conducted by Hong *et al.* (2005) indicated the usefulness of the *Alt a1* gene in establishing phylogenetic relationships of *Alternaria* species. This gene codes for analogs of an *A. alternata* protein, which can induce allergic responses in humans (Achatz *et al.* 1995; Simmon-Nobbe 2000; Weitchel *et al.* 2003). The nuclear gene coding for the glycolytic enzyme glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was also found to be useful in phylogenetic analyses of *Alternaria* species (Hong *et al.* 2005). More recently, the calmodulin gene has been also incorporated as a molecular tool for taxonomic and diversity studies of *Alternaria* species (Gannibal *et al.* 2014).

Early blight (EB) is an economically important *Alternaria*-induced disease of potato and tomato crops, which may affect leaves, stems, fruits, and tubers causing severe yield and quality losses (Strandberg 1992; Franc and Christ 2001; Simmons 2000, 2007; Jones 2014). Foliar susceptibility to EB pathogens increases as the tissues reach vegetative maturity and fungal damage is more severe in older (basal) leaves (Boiteux *et al.* 1995; Franc and Christ 2001; Jones 2014). The most

important EB-inducing *Alternaria* species described thus far belongs to the well-defined section *Porri* according to phylogenetic information and also morphometrical data, having as the main feature the presence of large, beaked conidia (Lawrence *et al.* 2013; Woudenberg *et al.* 2013; Gannibal 2015). *Alternaria solani* Sorauer has been historically described as the major EB-causing agent on tomato and potato crops worldwide (Henning and Alexandre 1959; Franc and Christ, 2001; Jones 2014; Adhikari *et al.* 2020), including Brazil (Boiteux *et al.* 1995; Lopes *et al.* 2005; Lourenço Jr. *et al.* 2009). However, *A. tomatophila* E.G. Simmons (Lawrence *et al.* 2013) was subsequently reported in more consistent association with tomato EB symptoms throughout the world (Simmons 2000; Gannibal *et al.* 2014). Afterwards, *A. grandis* E.G. Simmons was characterized as the main EB-inducing agent of potatoes (Simmons 2007; Lawrence *et al.* 2013). More recently, *A. tenuissima* was reported as the predominant pathogen causing foliar diseases of potato in China (75.5% of the isolates), followed by *A. alternata* (18.6%) and *A. solani* (5.9%) (Zheng *et al.* 2015). In this new taxonomic scenario, *A. solani* was characterized as being the only species of the genus able to cause EB on both potato (Zheng *et al.* 2015; Adhikari *et al.* 2020) and tomato crops (Simmons 2007).

Likewise, studies on the genetic variability of *A. solani* isolates from potatoes and tomatoes in Brazil indicated population subdivisions according to the host species, suggesting the potential involvement of a fungal complex as causal agents of EB in these two crops (Lourenço Jr. *et al.* 2009). Subsequent studies confirmed *A. tomatophila* as the major EB-inducing pathogen on tomatoes and *A. grandis* on potatoes in Brazil (Rodrigues *et al.* 2010). *Alternaria tomatophila* along with other *Alternaria* species reported as causal agents of EB on tomatoes such as *A. subcylindrica* E.G. Simmons & R.G. Roberts (Lawrence *et al.* 2013) and *A. cretica* E.G. Simmons & Vakal. (Lawrence *et al.* 2013) were latter synonymized as *A. linariae* (Neerg.) E.G. Simmons (Woudenberg *et al.* 2014). In addition, a distinct group of *Alternaria* species, some of them incorrectly identified (see Gannibal 2015) was reported to induce EB-like symptoms on distinct members of the Solanaceae family including: *A. alternata* (Fries) Keissler on potatoes (Boiteux and Reifschneider 1994) and tomatoes (Adhikari *et al.* 2020); *A. elegans* E.G. Simmons & J.C. David (Simmons 2000; Woudenberg *et al.* 2013), *A. mimicula* E.G. Simmons (Woudenberg *et al.* 2013), *A. tomaticola* (syn. *A. alternata*; Woudenberg *et al.* 2015) and *A. tomato* (Cooke) L.R. Jones on tomatoes; *A. crassa* (Sacc.) Rands E.G. Simmons (Hong *et al.* 2005; Lawrence *et al.* 2013) on bell-peppers (*C. annuum* L.), and *A. beringelae* E.G. Simmons on eggplants (Woudenberg *et al.* 2014).

As mentioned, previous studies combining morphological and molecular characterization have been conducted to clarify the diversity panorama of the EB-inducing *Alternaria* species on potato and tomato crops in Brazil (Lourenço Jr. *et al.* 2009; Rodrigues *et al.* 2010). However, a scarce amount of information is yet available about *Alternaria* isolates infecting a wide range of Solanaceae crops, weeds, and native/endemic species to the Neotropics. In this context, the main objective of this study was to characterize *Alternaria* isolates obtained from EB-like foliar lesions across all macro-geographical regions of Brazil on nine species in five solanaceous, including members of the native flora of the Neotropics.

Material and Methods

Fungal isolates

One hundred and twenty (120) monosporic *Alternaria* isolates were obtained across distinct geographic regions of Brazil (**Figure 1**) from five botanical genera and nine Solanaceae species (**Table 1**). These isolates were maintained in distilled sterilized water (Castellani 1939) as components of the “Collection of Fungi and Oomycetes” of the National Center for Vegetable Crops Research (CNPV). Samples of the reference *Alternaria* isolates, representing each clade/lineage obtained after the *Alt a1* phylogenetic analysis (see description below), were also deposited in the reference “Coleção de Fungos Fitopatogênicos – Professora Maria Menezes” (CMM), in Recife, Pernambuco State (PE), Brazil.

Fungal DNA extraction

Fungal isolates were recovered from distilled water and subcultured on Petri plates containing 10% V8 juice culture media and maintained in a growth chamber (25 °C / photoperiod of 12 hours) for seven days. After this time, under a laminar flow hood, the mycelium was removed from the plate with a scalpel and then placed into Precellys® tubes (Bertin Technologies, Ozyme, France) and stored in a deep freezer. DNA extraction was carried out by a CTAB/organic solvents method as described by Boiteux *et al.* (1999). Total purified DNA was subsequently stored in a freezer overnight and quantified in Lite NanoDrop® spectrophotometer (Thermo Scientific, Wilmington, DE, USA). Final concentrations were adjusted to 20 ng/μL by dilution in Tris-EDTA buffer.

Polymerase chain reaction (PCR) assays, primers, and target genomic regions – PCR assays were performed in a Veriti 96-Well Thermal Cycler® (Applied Biosystems, Foster City, CA,

USA) using specific primers for three genomic regions: The *A. alternata* allergenic protein-encoding gene (*Alt a1*), glyceraldehyde 3-phosphate-dehydrogenase (*GAPDH*), and calmodulin. Primers used in PCR assays for *Alt a1* gene were *Alt-rev* (5'-ACG AGG GTG AYG TAG GCG TC-3') and *Alt-for* (5'-ATG CAG TTC ACC ACC ATC GC-3') (Hong *et al.* 2005); the primer pair for amplifying the *GAPDH* region was *GPD-1* (5'-CAA CGG CTT CGG TCG CAT TG-3') and *GPD-2* (5'-GCC AAG CAG TTG GTT GTG C-3') (Berbee *et al.* 1999); and primers for the calmodulin gene amplification were *CaldF1* (5'-AGC AAG TCT CCG AGT TCA AGG-3') and *CaldR1* (5'-CTT CTG CAT CAT CAY CTG GAC G-3') (Lawrence *et al.*, 2013). PCR assays were performed using oligonucleotides from the IDT (Integrated DNA Technologies, Coralville, IA, USA) and the remaining reagents were from Invitrogen Life Technologies, diluted in Milli-Q® water (Millipore, Bedford, MA, USA). The final volume of the reaction was 20 µL. The mix of the *Alt a1* region was composed by the 1X buffer, 2.5 mM MgCl₂, 0.25 mM of each dNTP, 0.2 units recombinant *Taq* DNA polymerase, 20 nM each oligonucleotide, and 60 ng of purified genomic DNA. The PCR programs consisted of an initial denaturation step (at 94 °C for 5 minutes), followed by 35 amplification cycles (denaturing at 94 °C for 30 seconds, annealing at 64 °C for 1 minute, and extension at 72 °C for 13 seconds) with final extension step (72 °C for 10 minutes). The PCR program for amplifying the *GAPDH* region was established with an initial denaturation step at 94 °C for 5 minutes followed by 35 amplification cycles (denaturing at 94 °C for 30 seconds, annealing at 63 °C for 1 minute, and extension at 72 °C for 20 seconds) with a final extension step at 72 °C for 3 minutes. The mix for the amplification reaction of calmodulin region was adjusted to a final volume of 25 µL. The PCR cycles for calmodulin gene consisted of initial denaturation step at 95 °C for 4 minutes, 35 amplification cycles (denaturing at 95 °C for 30 seconds, annealing at 58.5 °C for 30 seconds, and extension at 72 °C for 13 seconds) with a final extension step at 72 °C for 5 minutes. The PCR products were subjected to electrophoresis on 1.5% agarose gels (w/v) impregnated with ethidium bromide. The amplicon images were captured with the EagleEye II® video system (Stratagene, La Jolla, CA, USA). PCR products were then gel-purified using the PureLink® (PCR Purification Kit Invitrogen K3100-02).

Sequencing characterization of the *Alternaria* isolates

Sequencing characterization of the isolates was subdivided in three steps in order to optimize experimental resources. In the first step, the *Alt a1* genomic regions of all the 120 isolates were sequenced. In the second step, a subset of 61 isolates (encompassing representative *Alt a1* haplotypes) was sequenced for the *GAPDH* genomic region. In the third step, the calmodulin

genomic region of 17 representative isolates of all the *Alt a1/GAPDH* clades were sequenced to provide additional information of phylogenetic relevance. Purified amplicons were sequenced with each primer used in the PCR assays. Sequencing was performed on an ABI PRISM sequencer (model 3100) at the Genomic Analysis Laboratory (CNPH), using the ABI PRISM BigDye® kit version 3.0 chemistry (Applied Biosystems).

Phylogenetic analyses

The quality of the sequences, contig assembly and sequence edition were performed in the SeqMan® program (Alex 1999). Alignments with sequences available at GenBank (**Table 2**) were carried out with the Megalign® program (Lasergene, Madison, WI, USA). The criteria employed to select the GenBank sequences used to build up the matrices were the following: accessions of *Alternaria* species reported on Solanaceae hosts and corresponding to reference isolates and/or *Alternaria* accessions deposited by *bona fide* taxonomic research groups. The program Geneious® version 8 (<http://www.geneious.com>) was used for aligning the *Alt a1*, *GAPDH* and calmodulin sequences and for Bayesian analyses. The fungal haplotypes were obtained by using the DnaSP 5:10 program (DNA Sequence Polymorphism). Parsimony and likelihood analyses were performed in the PAUP program [Swofford 2003. “Paup*: Phylogenetic analysis using parsimony (and other methods) 4.0. B5”]. The multiple sequence alignments of the *Alt a1*, *GAPDH* and calmodulin genes were performed with MAFFT algorithm (Kato and Standley 2013) as a plugin of Geneious Prime 2020. Bayesian, Maximum Likelihood and Maximum Parsimony analyses were initially performed separately (i.e., at a single gene basis). The substitution model for each gene was chosen with MEGA X software (Tamura et al. 2013) with Akaike information criterion. The maximum likelihood analyses of the *Alt a1* and *GAPDH* genes were carried out using the Hasegawa-Kishino-Yano Model (HKY) model (Hasegawa et al. 1985) with gamma distribution, invariable sites and 1,000 bootstraps, run with PHYML plugin. The maximum likelihood analysis of the calmodulin gene was done using the GTR (General time reversible) substitution model (Tavaré 1986), with gamma distribution and 1,000 bootstraps, run with PHYLM plugin. The maximum parsimony analysis was carried out on PAUP 4.0 (Swofford 2003) using random step addition as parameter (1,000 replicates) with branch swapping and tree bisection-reconnection. The Bayesian inference analyses of the *Alt a1* and *GAPDH* genes were performed on MrBayes (Huelsenbeck and Ronquist 2001) plugin (version 3.2.2) in Geneious Prime 2020 with HKY model with 2,000,000 and 10,000,000 generations chain, respectively. Bayesian analysis of the calmodulin gene was carried out employing MrBayes (Huelsenbeck and Ronquist 2001) plugin

(version 3.2.2) in Geneious Prime 2020 with GTR substitution model (Tavaré 1986), with gamma distribution, 2,000,000 generations chain, subsampling each 100 and burn-in of 25%. The *A. mimicula* reference sequence BMP 0324 was employed as the out-group in the analyses using the *Alt a 1* and *GAPDH* genomic regions. The *A. mimicula* sequence EGS 01.056 was employed as the out-group in the analysis of the sequences derived from the calmodulin-coding gene. Alignment matrices and analyses were deposited in TreeBase (<http://purl.org/phylo/treebase/phyloids/study/TB2:S27825?x-access-code=d4ebf1c23b71717909a77dad49769f51&format=html>).

Morphological characterization of 17 *Alternaria* isolates representative of the observed phylogenetic groupings

Eight isolates from tomato, four from potato, one from eggplant, and four isolates from solanaceous weeds were selected for further morphological characterization based upon the diversity groups formed in the previously described molecular analyses. For morphological characterization, the isolates were at first grown on Petri plates containing either V8 Campbel® juice 17.5% (pH adjusted to 6.4) or potato carrot agar (PCA). Petri plates were maintained in growth chambers at 22 °C for seven days. The isolates were then transferred to Petri plates containing either V8 media (pH 6.4) or potato carrot agar (PCA) without parafilm sealing followed by an incubation step in a growth chamber (22 °C/photoperiod of 8 hours of white light and 16 hours dark) for 5 to 7 days (Simmons 2007). *Alternaria* spores were used in the microscopic preparations. Conidial measurements were taken under an optical microscope (400 X) from the length and width of the conidial body and conidial rostrum length (50 conidia by isolate). It was also quantified the number of transverse and longitudinal septa, and the rostrum numbers.

Pathogenicity and host range assays employing a selected subgroup of 13 *Alternaria* isolates

Thirteen isolates, molecularly and morphologically characterized in previous sections of the present work were used in this study (**Table 3**). Seven isolates were obtained from tomato, one from potato, one from eggplant, one from scarlet eggplant, one from thorn apple (*Nicandra physalodes* L.), one from jimson weed (*Datura stramonium* L.), one from wolf-fruit (*S. lycocarpum*), and two from tobacco (*Nicotiana tabacum*). These isolates were initially cultivated on Petri plates containing either V8 media (pH 6.4) or potato carrot agar (PCA). Petri plates were

kept with no parafilm and maintained in growth chambers at 22 °C for seven days (Simmons, 2007). After this period, 10 mL of distilled sterilized water plus 0.1% of Tween 80® was added to each plate and the conidia were removed by superficial scraping of the colony with soft bristle brush. The suspension was filtered through sterile double layer gauze and collected in a beaker. The quantification of the number of conidia in the suspension of each isolate was made with the help of a hemocytometer and the concentrations were adjusted to 10⁴ conidia per mL. Plants of seven solanaceous hosts were inoculated with a subset of 11 isolates viz. tomato, potato, pepper (*C. annuum* L.), eggplant, scarlet eggplant, jimson weed, and the leaf ground cherry (*Physalis angulata* L.). The two *Alternaria* isolates from tobacco were inoculated only on tobacco plants. Seeds were sowed in 128 cell trays containing Plantmax® substrate, placing two seeds per cell, which were kept under greenhouse conditions. After 15 days the seedlings were transferred to 1-L pots containing sterilized soil and being kept under the same conditions previously mentioned. Two months later, aerial parts of the plants were sprayed individually with each conidial suspension. Inoculated plants were placed in a moist chamber for 24 hours. The evaluation of the presence or absence of characteristic EB lesions was made 7–10 days after inoculation. Two leaves of each plant with typical EB symptoms were collected and placed in a moist chamber aiming to confirm the sole presence of the inoculated pathogen in the lesions. From these leaves, fungi were re-isolated in water agar media. Isolates were considered to be pathogenic only if they were able to induce lesions and sporulation on inoculated leaves. A completely randomized experimental design was employed with three replicates per treatment. Each replicate was represented by one pot with two plants each. This assay was carried out twice.

Results

Phylogenetic analyses using the *Alt a1* gene region

The phylogenetic analyses based upon Bayesian inference, Maximum Parsimony, and Maximum Likelihood of *Alt a1* region of 120 *Alternaria* isolates are shown in the **Figure S1** and **Table 1**. Sixty-one *Alt a1* haplotypes were identified in this initial analysis (**Figure 2**). The assembly of the contigs from the *Alt a1* haplotype region (within the 428 bp) and their alignments (after trimming based on sequence quality) yielded sequences of 307 characters. From those, 299 were constant; 107 were variable; 73 were parsimony-informative. All analyses produced trees with the same topology. Due to the high levels of similarities of trees the bootstrap values of maximum likelihood, maximum parsimony from the sequence analysis were transferred to the Bayesian

inference tree. The catenulated species *A. minicula*, which belongs to the distinct *Alternaria* section Brassicicola (Woudenberg *et al.* 2013) was found to be a proper out-group as indicated by its clear segregation from the specimens under analysis in our study. The phylogenetic tree of the *Alt a1* haplotypes was composed by six major groups of isolates. The first group was composed of 16 isolates from *S. tuberosum*, two from *S. melongena* and one from *N. physalodes* plus the reference *S. tuberosum* isolates of *A. grandis* (CBS109158) and *A. solani* (EGS 45020), with 0.96 posterior probability (PP) and bootstraps of 88% and 93% for Maximum Parsimony (MP) and Maximum Likelihood (ML), respectively. The second group was composed of two *S. lycopersicum* isolates (EH-862 and EH-1210), one isolate from *C. annuum* (EH-1406) and two isolates from *N. physalodes* (EH-1654 and EH-1655) plus the reference isolates of *A. argyroxiphii* (EGS35-122 from unknown host and PPRI 11848, a South African isolate from *Ipomoea batatas*), with 0.95 PP and bootstraps of 66% and 67% for MP and ML, respectively. The third group was formed by the reference *A. dauci* isolates (BA1491 and BMP 0167), one isolate from *S. lycopersicum* (EH-1143) and one isolate from *Datura stramonium*. This group displayed PP of 1 and bootstraps of 95% and 93% for MP and ML, respectively. The fourth group included 15 isolates from *S. lycopersicum*, one from *S. melongena*, one from *S. commersonii* and one from *S. lycocarpum*, plus the reference isolates of *A. tomatophila* (CBS109156), *A. cretica* (CBS109164) and *A. subcylindrica* (CBS109161), all of which have recently been synonymized as *A. linariae*, *A. ciphomandrae* (CBS109155, isolate from *S. betaceae* in New Zealand) and *A. solani-nigri* (CBS113403, isolate from *S. nigrum* in India). This group displayed PP value of 1 and bootstraps of 98% for MP and 100% ML. The fifth group was composed of three isolates from *S. lycopersicum* (EH-1120, EH-1258 and EH-1437), one from *S. tuberosum* (EH-1185), one from *D. stramonium* (EH-1925) and one from *C. annuum* (EH-1168), plus the reference isolates of *A. arborescens* (CBS102605) and *A. alternata/A. tenuissima* (CBS113013). This group was supported by high (1.0) probability and bootstrap values ranging from 83 to 92 for MP and from 95 to 97 for ML. The sixth group was composed of two isolates (EH-1489 and EH-1495) from *N. tabacum* and clustered with the reference *A. longipes* isolates. The present phylogenetic analysis using information derived only from the *Alt a1* gene region was not able to discriminate isolates of *A. solani*, *A. linariae* and *A. grandis*. A subgroup of the reference isolates clustered with *A. grandis* and others with *A. linariae*. However, most of the *S. tuberosum* isolates grouped with the reference isolates of *A. grandis*, whereas most of the *S. lycopersicum* isolates grouped with the reference isolates of *A. linariae*. The EH-1435 and EH-1261 isolates from tomato formed a

subgroup with close relationship with the *A. linariae* group, supported with 1.0 values of PP and bootstraps of 98% for MP and 100% for ML. The same was observed with the EH-1559 and EH-1180 isolates (both from *S. tuberosum*) that formed a cluster related to the *A. grandis* group. The other isolates were grouped randomly in groups regardless of the host of origin and geographic region. One of these groups displayed close relationship to *A. dauci* and *A. argyroxiphii*. The remaining isolates (EH-0768, EH-1208, EH-1252, EH-1381, EH-1806, EH-2113, and EH-2115) were not allocated to any of the previous groups.

Phylogenetic analyses of *GAPDH* gene region of a subset of 61 isolates representing distinct *Alt a1* haplotypes

The *GAPDH* genomic region of a subset of 61 isolates encompassing *Alt a1* haplotypes described above were also sequenced aiming to refine our analyses. The phylogenetic tree of *GAPDH* gene based upon Bayesian inference, Maximum Parsimony and Maximum Likelihood of this subgroup of selected isolates is shown in **Figure 3**. From the obtained *GAPDH* sequence alignments of 925 bp, 47 nucleotides were parsimony-informative, 73 were variable sites, 414 were conserved sites, yielding a sequence of 487 characters. Due to the high levels of similarities of trees, the bootstrap values of ML and MP from the sequence analysis were transferred to the Bayesian inference tree. The bootstrap values and posterior probabilities that support the *GAPDH*-derived subdivision of groups 'randis', 'cretica', 'linariae', 'subcylindrica', 'dauci', and 'crassa' were low. These analyses were carried out several times aiming to increase the values and they always showed similar levels of support. However, even with these low support values for the *GAPDH* sequence information, the *Alt a1* and calmodulin genetic information from a select group of isolates in association with morphometrical and pathogenicity data gives support to the subdivision of the 'grandis', 'cretica', 'linariae', 'subcylindrica', 'dauci', and 'crassa' into distinct groups. The first group was composed of 17 isolates from *S. tuberosum*, 13 from *S. lycopersicum*, two from *D. stramonium* (EH-1925 and EH-1926) and four (EH-0679, EH-0680, EH-1509, and EH-1806) from *S. melongena* and one (EH-1952) from *S. commersonii* grouped with reference isolates of *A. grandis*, with 0.80 PP, 62% for MP and ML. The second group was composed of six *S. lycopersicum* isolates (EH-1435, EH-1792, EH-1823, EH-1836, EH-1174, and EH-1261), one *S. lycocarpum* isolate (EH-1642), and one *N. physalodes* isolate (EH-2116), which clustered with reference *A. linariae* isolates from tomatoes. The isolate EH-1143 was again clustered in the *A. dauci* group, being supported with 0.9 values for PP, 61% for MP and 63% for ML. The isolates

EH-0862, EH-1214, EH-1406, EH-1654, and EH-1655 were assigned as *A. argyroxiphii*, displaying PP value of 1, 96% for MP and 95% for ML. The isolate EH-1252, obtained from tomato in the Federal District was clustered with the reference isolates of *A. crassa* (syn. *A. capsici*), displaying PP value of 1, 94% for MP and 92% for ML. The isolates EH-1489 and EH-1495 from tobacco were again clustered in the *A. longipes* group, being supported by high probability and bootstrap values. Likewise, the isolates EH-1120 and EH-1437 from *S. lycopersicum*, the isolates EH-1168 from *C. annuum* and EH-1185 from *S. tuberosum* were grouped again with species of the *A. alternata*/*A. tenuissima* /*A. arborescens* complex (**Fig. 3**).

Phylogenetic analyses of the calmodulin genomic region of a subset of 17 *Alternaria* isolates

The calmodulin genomic region of 17 representative isolates of the previously described *Alt al*/*GAPDH* clades were also sequenced to provide additional information of phylogenetic relevance (**Figure 4**). The calmodulin sequence alignments generated 1493 bp with 100 nucleotides being informative, 177 were variable, 452 were constant, yielding a sequence of 665 characters. Due to the high levels of similarities of trees the bootstrap values of maximum likelihood, maximum parsimony from the sequence analysis were transferred to the Bayesian inference tree. It was possible to identify in this set of analyses four *A. grandis* isolates, three from potato (EH-0809, EH-1180, and EH-1548) and one from eggplant (EH-0679), being supported by high probability (1.0) and bootstrap values 96% for MP and 92% for ML. The tomato isolates (EH-1261, EH-1435, EH-1823, and EH-1836) and the *S. lycocarpum* isolate (EH-1642) clustered together with *A. subcylindrica*, and *A. tomatophila* (= *A. linariae*). The isolates EH-0862, EH-1214, EH-1406, EH-1654, and EH-1655 displayed close genetic relationship with *A. argyroxiphii*, which agrees with the former trees. The isolate EH-1143 (from *S. lycopersicum*) clustered with the reference *A. dauci* isolate (BMP 167), whereas the *S. lycopersicum* isolate EH-1252 clustered with *A. crassa* (as also observed in previous analyses).

Morphological characterization

The description of the morphological traits of eight *Alternaria* isolates from tomato, four from potato, one from eggplant, and four from other Solanaceae species is shown in **Table 4**. Morphological characterization of the potato infecting isolates EH-0809, EH-1180, and EH-1548 were in agreement with that of *A. grandis*. The tomato infecting isolates (EH-1261, EH-1435, and EH-1823) and the isolate EH-1642 from *S. lycocarpum* were identified as *A. linariae*, which is in accordance with the molecular characterization. The isolates EH-1143 from tomato, and EH-0679 from eggplant were molecularly identified as *A. dauci*, and *A. grandis*, respectively. However, the

morphological data of their conidia were somewhat divergent from the original description of the corresponding reference isolates of these species (Table 4).

Host range/pathogenicity assays

Host range evaluation of a selected group of 13 isolates is shown in Table 3. Profuse fungal sporulation was observed in foliar EB-like lesions of all symptomatic plants under moist chamber conditions and all isolates were re-isolated in pure culture. Eleven isolates induced EB-like symptoms in tomato independent of their original hosts. Seven out of 11 isolates were able to induce typical EB symptoms in potato, being three from tomato, one from potato, one from eggplant, one from jimson weed, and one from wolf-fruit. The isolate EH-1642 from wolf-fruit (identified as *A. linariae*) induced EB symptoms on all host plants included in the test, except sweet-pepper. Isolates characterized as *A. linariae* (EH-1823) and *A. grandis* (EH-1548) from tomato and potato, respectively, showed no specificity for their original hosts. The EH-1823 isolate was able to induce EB-like lesions on tomato and potato, while the EH-1548 induced typical EB symptoms on tomato, potato, and scarlet eggplant. The tomato-infecting isolate EH-1143 (*A. dauci*) displayed a host-specific behavior, causing EB-like symptoms only on tomato. The isolates EH-1214 (*A. argyroxiphii*), EH-1252 (*A. crassa*), and EH-2113 (*Alternaria* sp.) from tomato and EH-0679 (*A. grandis*) from eggplant were able to cause typical EB lesions in *D. stramonium* and *P. angulata*, two widespread solanaceous weeds in the neotropical regions. Intriguingly, the isolates EH-1252 and EH-1738 (*A. crassa* = *A. capsici*) displayed distinct host ranges and they were not able to induce symptoms in *C. annuum* in our assays. The isolates EH-1489 and EH-1495 (from *N. tabacum*) were inoculated only in tobacco plants and both were able to induce foliar lesions.

Discussion

The molecular diversity of EB-inducing *Alternaria* species has been investigated mainly on potato and tomato crops in Brazil (Lourenço Jr. *et al.* 2009; Rodrigues 2009; Rodrigues *et al.* 2010). However, more extensive studies about *Alternaria* isolates infecting a wide range of Solanaceae species native/endemic to the Neotropics was still lacking in the literature. Here, we conducted phylogenetic analyses of three genomic regions from a representative set of fungal isolates of the genus *Alternaria* obtained from nine Solanaceae host species. Our first molecular tool for fungal species identification and to establish phylogenetic relationships among all the 120 *Alternaria* isolates of this collection was the employment of *Alt a1* gene-derived sequence information, since this genomic region provides satisfactory number of informative sites with good resolution levels

(Hong *et al.* 2005). However, in our study, the phylogeny of *Alt a1* gene region alone displayed limited value for distinguishing closely related species such as *A. grandis* and *A. solani*. This can be explained by the relative low number of mutations in *Alt a1* gene observed across EB-inducing *Alternaria* species associated with Solanaceae members is not enough to allow a robust phylogenetic discrimination within this closely-related group of pathogens. These results agree with Lourenço Jr. *et al.* (2009) that also found a relatively low number of polymorphisms in the *Alt a1* gene sequence among EB-inducing isolates from potato and tomato. In fact, a growing number of studies has showed that the low sequence variability of some selected genomic regions makes the discrimination of some closely related *Alternaria* species very difficult (Berbee *et al.*, 2003; Gannibal *et al.*, 2014; Woudenberg *et al.*, 2014). Therefore, a multi-locus phylogenetic approach has been implemented with the inclusion of DNA sequences of another gene regions in order to allow for a more precise discrimination of closely related species such as *A. grandis*, *A. solani*, and *A. linariae* (Gannibal *et al.* 2014; Woudenberg *et al.* 2014). Our work allowed the report of a series of novel host-pathogens interactions including *A. grandis* isolates infecting *D. stramonium*, *S. melongena*, and *S. commersonii*; *A. dauci* isolates on tomato; *A. linariae* isolates in *N. physalodes* and in the neotropical species *S. lycocarpum*; and *A. argiroxiphii* isolates in tomato, *C. annuum*, and *N. physalodes*. The occurrence of *A. alternata* species complex isolates causing foliar lesions in tomato, potato, and *C. annuum* and *A. longipes* isolates in *Nicotiana tabacum* were also molecularly confirmed. In previous studies, *Alternaria* isolates from tomato and potato were discriminated with high support values by phylogenetic analyses using concatenated *Alt a1*, *GAPDH*, and calmodulin gene sequences (Gannibal *et al.* 2014). In fact, most of our tomato isolates were placed into the *A. linariae* cluster, whereas most of the potato isolates were grouped in the *A. grandis* lineage. However, *A. linariae* isolates in natural association with potato and *A. grandis* with tomato were also observed. As mentioned, one *A. linariae* isolate (EH-1642) was found in association with foliar lesions on wolf-fruit. One working hypothesis is that cultivated tomato might be the original source of *A. linariae* inoculum, since the EH-1642 isolate from wolf-fruit was collected in Ibicoara-BA, one of the main regions of tomato production of the Northeast Brazil. Similar situation was observed with tomato isolates of *Stemphylium solani* that were able to infect nearby wolf-fruit plants in Central Brazil (Boiteux *et al.* 1993b). The lack of host specificity of the isolate EH-1642 is not in agreement with the information indicating a more restricted host range of *A. linariae* (Rodrigues *et al.*, 2010). On the other hand, *A. solani* is able to infect a wider host range under different environmental conditions (Bessadat *et al.* 2017).

Intriguingly, *A. solani* and its closely related species *A. protenta*, two common species reported infecting solanaceous hosts in other countries (Woudenberg *et al.* 2014; Landschoot *et al.* 2017; Ayad *et al.* 2019; Adhikari *et al.* 2020; Farr and Rossman 2020) were not found in our study, even though it has been demonstrated that *A. solani* can cause disease in potato and in at least four different *Solanum* species under field conditions (Gannibal *et al.* 2014; Adhikari *et al.* 2020). This absence of both species under Brazilian conditions remains elusive. Historically, *A. solani* was wrongly assigned as the single causal agent of EB on tomato and potato in Brazil (Boiteux *et al.* 1995; Lopes *et al.* 2005; Lourenço Jr. *et al.* 2009). Two hypotheses can explain this somewhat surprising absence of *A. solani* in Brazil: **(1)** this fungal species was present in the country but it was gradually replaced by more adapted isolates of *A. grandis* in potato and *A. linariae* in tomato and **(2)** the previous reports of *A. solani* are more likely erroneous descriptions of this species due to the fact that they were based solely upon morphological characteristics that do not allow an accurate discrimination of *A. solani*, *A. grandis*, and *A. linariae* isolates. A series of independent studies is showing that, in general, some dematiaceous hyphomycetes infecting the same host plant tend to belong to the same phylogenetic group independent of their geographic origin (Rodrigues *et al.* 2010; Brun *et al.* 2013; Groenewald *et al.* 2013; Manamgoda *et al.* 2014; Gannibal *et al.* 2014; Woudenberg *et al.* 2014). Multi-locus analyses using concatenated phylogeny of ITS, *GAPDH*, *RPB2*, *TEF1*, and *Alt a1* genomic regions have allowed the identification of *Alternaria* species, which were also separated into different groups according to their original hosts (Woudenberg *et al.* 2014). Other studies have also detected the occurrence of host-dependent phylogenetic grouping in concatenated analyses of *Alt a1*, *GAPDH*, and ITS regions. One hypothesis for the formation of closely related groups according to host species may occur due to the putative presence of evolutionarily conserved DNA motifs that are required for the establishment of specific *Alternaria*-host species interactions. Here, concatenated phylogenies were not carried out due the lack of GenBank reference accessions with genomic information for all the three genes. However, in our work, only a partial host-guided phylogenetic grouping was observed, with isolates from the same Solanaceae species not always clustering together for all three genomic regions. It was recently found in the three phylogenetic analyzes the occurrence of random groups comprising *Alternaria* section Porri isolates from different hosts and geographic regions (Ozkilinc *et al.* 2018), which agrees with our observations with a subset of *A. grandis*, *A. linariae*, *A. argyroxiphii* isolates. According to Woudenberg *et al.* (2014) *A. linariae* (= *A. tomatophila*) and *A. grandis* have close phylogenetic relationships and they also display an overlap

in their host ranges comprising species of the same botanical genus. This would explain the fact that some *A. linariae* and *A. grandis* isolates are pathogenic to both tomatoes and potatoes, especially when using controlled inoculation with high inoculum pressure under temperature and moisture conditions favorable to infection. This observation is in accordance with the results described by Rodrigues (2009) in which host specificity behavior of *A. tomatophila* and *A. grandis* isolates is not universally observed. Therefore, isolates of *A. grandis* (a major potato pathogen) able to cause disease on tomato and *A. linariae*, which is a major tomato pathogen can also cause EB on potato. It is interesting to highlight that our *A. linariae* isolate EH-1823 was more aggressive to both tomato and potato when compared to the one *A. grandis* (EH-1548) isolate (data not shown). However, this observation needs more extensive studies to be confirmed. It is possible that a subset of our isolates that were not grouped with any of the reference probably belong to either different species or to novel species not yet reported infecting Solanaceae hosts. The *Alt a1*, *GAPDH*, and calmodulin were useful phylogenetically once they provide high supported groups (Lawrence *et al.* 2013). The same pattern was observed in our phylogenetic analysis, reinforcing the possibility that the groups discriminated from reference isolate may be either other reference species (not included in our analyses) or even new species, as these groups have been supported by high posterior probability values and bootstraps. Two isolates EH-1252 and EH-1738 were found to belong to the same cluster of *A. crassa*. However, EH-1252 may be either a variant of this species or new species with close genetic relationship to *A. crassa*. Therefore, this collection of atypical isolates should be better characterized biologically, morphologically, and molecularly to allow for a more precise identification at species level. The species *A. dauci* was first described by Groves and Skolko (1944) and it is the major causal agent of the carrot (*Daucus carota* L.) leaf blight under Brazilian conditions (Boiteux *et al.* 1993a). Simmons (1995, 2007) characterized *A. dauci* via morphological attributes and by confirming its pathogenicity to carrot. Phylogeny studies of *A. dauci* isolates have confirmed the morphological characterization of this species and its ability to cause leaf blight on carrot and coriander *Coriandrum sativum* L. both from the Apiaceae family (Brun *et al.* 2013; Woudenberg *et al.* 2014). *Alternaria dauci* isolates have been also recovered from naturally infected celery (*Apium graveolens* L., Apiaceae) and lettuce (*Lactuca sativa* L., Asteraceae) leaf spots (Koike *et al.* 2017). Two *A. dauci* isolates from France were able to infect and induce mild foliar symptoms in tomatoes under artificial inoculation conditions (Boedo *et al.* 2012). However, to our knowledge, no information is available about *A. dauci* isolates infecting members of the Solanaceae family

under natural conditions. In the present study, the isolate EH-1143 obtained from tomato in Rio Grande do Sul State was phylogenetically identified as *A. dauci* and it was able to induce EB-like symptoms on tomatoes in our pathogenicity bioassays. Thus, our work represents the first formal report of an *A. dauci* isolate causing EB on tomato plants in Brazil. It is important to point out that this isolate was detected in a rural area where tomato and carrot crops are cultivated side-by-side. It will be of interest to further investigate the host range of EH-1143 in Apiaceae, Asteraceae, and Solanaceae species aiming to identify the potential presence of *A. dauci* isolates with host-specific interactions. *Alternaria argyroxiphii* E.G. Simmons and Aragaki (Lawrence *et al.* 2013) was morphologically characterized by Simmons (1997) and it is currently allocated in Porri section (Woudenberg *et al.* 2014). This species was first described causing leaf spots on *Ipomoea batatas* in South Africa. However, only *A. bataticola* isolates have been reported in association with *I. batatas* in Brazil (Lopes and Boiteux 1994). In fact, the first molecular characterization of *A. argyroxiphii* isolates in the country was carried out by Teixeira *et al.* (2017) in association with leaf spots on African mahogany trees [*Khaya senegalensis* (Desv.) A. Juss, Meliaceae]. However, no *A. argyroxiphii* isolate was found thus far infecting either tomatoes or other Solanaceae species. Therefore, our work is also the first record of *A. argyroxiphii* as a pathogen of tomato, sweet-pepper, and *N. physalodes* in the country and in world.

Strandberg (1992) found that *A. alternata* isolates showed no clear host specific patterns, causing foliar lesions on tomatoes, potatoes, *Capsicum* spp., and eggplant. This observation is in agreement with our phylogenetic results that indicated that distinct isolates from the *A. alternata* species complex (viz. EH-1185, EH-1381, EH-1437, and EH-1168) were able to cause foliar lesions on tomato, potato, and sweet pepper. Our results agree with reports indicating that isolates from the *A. alternata* species complex can cause symptoms similar to early blight on a wide range of solanaceous hosts (Landschoot *et al.* 2017; Abbo *et al.* 2018; El Gobashy *et al.* 2018; Adhikari *et al.* 2020; Farr and Rossman 2020; Mendes and Urban 2020). The isolates EH-1489 and EH-1495, obtained from tobacco (*Nicotiana tabacum* L.) leaf spots in Santa Catarina state (South Brazil), were identified as *A. longipes* according to the phylogeny of the *Alt a1* and *GAPDH* gene regions. Although these isolates were not fully morphologically characterized, we observed that they have short conidia that were produced in chains, typical of the species *A. longipes* (data not shown). There was only an old report of this disease in southern Brazil, in the state of Rio Grande do Sul (Costa Neto 1937). Although there is no record of the occurrence of this fungus in Brazil in

the database of plant pathogenic fungi of Embrapa Cenargen (Mendes and Urban 2020), other literature already pointed out this fungus as the causative agent of the disease in Brazil (Farr and Rossman 2020). However, this is the first report of the association of this fungus with tobacco leaf spots confirmed via molecular approaches. The ecological and epidemiological consequences of pathogens displaying wide host range when compared with the host-specific ones are related to their increased ability to subsist under natural conditions, becoming a persistent source of inoculum to potential epidemics. The absence of host-specificity has been observed across many *Alternaria*-host pathosystems. For example, Brazilian isolates of *A. brassicae* and *A. brassicicola* obtained from distinct Brassicaceae hosts were able to cause foliar lesions under greenhouse conditions in wide range of species and genera within this botanic family (Reis and Boiteux 2010). The survival of *A. cucumerina* isolates in watermelon (*Citrullus lanatus* Thunb) as well as on a range of wild cucurbits has been also observed (Kucharek 2000; Batista *et al.* 2009). Likewise, the results of our study indicated that persistent weeds such as *N. physalodes*, *D. stramonium* and *P. angulata* may act as alternative hosts of *Alternaria* species pathogenic to tomato and potato crops. The isolates EH-1654 (= *A. argyroxiphii*) obtained from *N. physalodes* caused EB symptoms on tomato, the isolate EH-1738 (= *A. crassa*) obtained from *D. stramonium* caused EB in tomato, potato and scarlet eggplant, and the isolate EH-1642 from *S. lycocarpum* (= *A. linariae*) caused EB symptoms on tomato, potato, eggplant and scarlet eggplant, indicating that some native and some invasive solanaceous species can act as source of inoculum of *Alternaria* species pathogenic to a wide range of economically important crop species.

The morphological characteristics of the conidia for most of the evaluated isolates identified in this study were similar to those described by Simmons (2007) for each corresponding *Alternaria* species. In a research work carried out in Brazil combining morphological and molecular characterization of *Alternaria* isolates from tomato and potato, Rodrigues *et al.* (2010) distinguished *A. tomatophila*, *A. cretica* (both synonymized as *A. linariae*) and *A. grandis* and also a representative isolate of *A. solani* by morphological characteristics. Gannibal *et al.* (2014) were able to distinguish morphologically *A. tomatophila* from *A. solani* when studying several *Alternaria* isolates from Russia. However, according to Van der Walls *et al.* (2004), populations of *A. solani* have high genetic variability, which can reflect in distinct biological and morphological characteristics as previously observed by Rotem (1994). In fact, this ‘morphological plasticity’ is probably not restricted to *A. solani* but may also be present among

isolates of other species from the section Porri such as *A. dauci*, *A. grandis* and *A. linariae* (which were well identified by phylogeny in our work). Therefore, it is possible that this yet cryptic genetic variability might cause the morphological discrepancies observed for the isolates EH-1143 (= *A. dauci*), EH-0679 (= *A. grandis*), and EH-1642 (= *A. linariae*) when compared to the reference isolates. The observed biological and taxonomic diversity of the *Alternaria* isolates causing EB on across Solanaceae species, indicated the potential implications of these hosts as sources of inoculum for economically important crops such as tomato and potato. Research combining molecular and morphological characterization for the identification of *Alternaria* species of Solanaceae and evaluation of the range of solanaceous hosts of the pathogen in Brazil are still relatively scarce in the literature (Lourenço Jr. *et al.* 2009; Rodrigues *et al.* 2010). In this context, the present work is a new contribution about characterizing the phenotypic and genetic diversity of *Alternaria* isolates causing EB in Solanaceae species, providing the scientific basis of efficient cultural management systems of this disease across different production systems in Neotropical areas. The observed diversity and host-specificity patterns of the *Alternaria* isolates from Solanaceae species may also have practical implications in establishing effective EB breeding for resistance strategies, especially for tomato and potato crops.

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Compliance with ethical standards

Disclosure of potential conflict of interest – The authors do not have any conflict of interest.

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Authors contribution statement

Ailton Reis and Celma C. Peixoto collected the fungal isolates. Ailton Reis, Leonardo Boiteux and Maria Esther planned the research and coordinated the laboratories involved in the research. Celma C. Peixoto and Cléia Cabral performed the experiments and Lab work. This work is part of the research for Celma C. Peixoto's doctoral thesis. All authors contributed to the writing and revision of the manuscript.

Table 1 List of 120 *Alternaria* isolates used in the present work with their original Solanaceae hosts, geographic location, and year of collection

Isolate code ^a	Original host	Geographical location ^b	Year
EH-0007	<i>N. physalodes</i>	Gama-DF	2002
EH-0014	<i>S. lycopersicum</i>	Lago Azul-GO	2002
EH-0568	<i>S. lycopersicum</i>	Lavras-MG	2003
EH-0584	<i>S. lycopersicum</i>	Recanto das Emas-DF	2003
EH-0643	<i>S. tuberosum</i>	Mucugê-BA	2003
EH-0679	<i>S. melongena</i>	Teixeiras-MG	2003
EH-0680	<i>S. melongena</i>	Ibipeba-BA	2003
EH-0686	<i>D. stramonium</i>	Irecê-BA	2003
EH-0694	<i>S. tuberosum</i>	Gama-DF	2003
EH-0742	<i>S. lycopersicum</i>	Barbacena-MG	2004
EH-0768	<i>S. lycopersicum</i>	Cristópolis-BA	2003
EH-0809	<i>S. tuberosum</i>	Cristalina-GO	2003
EH-0862	<i>S. lycopersicum</i>	Cristalina-GO	2003
EH-1120	<i>S. lycopersicum</i>	Alfredo Wagner-SC	2005
EH-1122	<i>S. lycopersicum</i>	Lebon Régis-SC	2005
EH-1143	<i>S. lycopersicum</i>	Pelotas-RS	2005
EH-1168	<i>C. annuum</i>	Camocim de São Félix-PE	2005
EH-1174	<i>S. lycopersicum</i>	Araucária-PR	2005
EH-1176	<i>S. tuberosum</i>	Contenda-PR	2005
EH-1180	<i>S. tuberosum</i>	Contenda-PR	2005
EH-1182	<i>S. tuberosum</i>	Contenda-PR	2005
EH-1183	<i>S. tuberosum</i>	Contenda-PR	2005
EH-1185	<i>S. tuberosum</i>	São Francisco de Paula-RS	2005
EH-1186	<i>S. tuberosum</i>	São Francisco de Paula-RS	2005
EH-1189	<i>S. tuberosum</i>	São Francisco de Paula-RS	2005
EH-1208	<i>S. lycopersicum</i>	Colombo-PR	2005
EH-1209	<i>S. lycopersicum</i>	Araucária-PR	2005
EH-1210	<i>S. lycopersicum</i>	Araucária-PR	2005
EH-1214	<i>S. lycopersicum</i>	Gama-DF	2005
EH-1228	<i>S. lycopersicum</i>	Gama-DF	2005
EH-1230	<i>S. tuberosum</i>	Major Vieira-SC	2005
EH-1232	<i>S. tuberosum</i>	Major Vieira-SC	2005
EH-1233	<i>S. tuberosum</i>	Major Vieira-SC	2005
EH-1234	<i>S. tuberosum</i>	Papanduva-SC	2005

EH-1235	<i>S. tuberosum</i>	Papanduva-SC	2005
EH-1236	<i>S. tuberosum</i>	Papanduva-SC	2005
EH-1237	<i>S. tuberosum</i>	Canoinhas-SC	2006
EH-1238	<i>S. tuberosum</i>	Canoinhas-SC	2006
EH-1239	<i>S. tuberosum</i>	Canoinhas-SC	2006
EH-1249	<i>S. tuberosum</i>	Pelotas-RS	2005
EH-1252	<i>S. lycopersicum</i>	Itobi-SP	2005
EH-1255	<i>S. lycopersicum</i>	Planaltina-DF	2005
EH-1256	<i>S. lycopersicum</i>	Planaltina-DF	2005
EH-1257	<i>S. lycopersicum</i>	Planaltina-DF	2005
EH-1258	<i>S. lycopersicum</i>	Planaltina-DF	2005
EH-1261	<i>S. lycopersicum</i>	Águas Claras-DF	2005
EH-1266	<i>S. tuberosum</i>	Gama-DF	2005
EH-1330	<i>S. tuberosum</i>	Guaraciaba do Norte-CE	2005
EH-1331	<i>S. tuberosum</i>	Guaraciaba do Norte-CE	2005
EH-1332	<i>S. tuberosum</i>	Guaraciaba do Norte-CE	2005
EH-1336	<i>S. tuberosum</i>	Guaraciaba do Norte-CE	2005
EH-1339	<i>S. tuberosum</i>	Guaraciaba do Norte-CE	2005
EH-1341	<i>S. tuberosum</i>	Guaraciaba do Norte-CE	2005
EH-1374	<i>S. lycopersicum</i>	Guaraciaba do Norte-CE	2005
EH-1376	<i>S. lycopersicum</i>	Guaraciaba do Norte-CE	2005
EH-1381	<i>S. lycopersicum</i>	Capão Bonito-SP	2005
EH-1406	<i>C. annuum</i>	Igarapé-MG	2006
EH-1435	<i>S. lycopersicum</i>	Itapetininga-SP	2005
EH-1436	<i>S. lycopersicum</i>	Itapetininga-SP	2005
EH-1437	<i>S. lycopersicum</i>	Itapetininga-SP	2005
EH-1440	<i>S. lycopersicum</i>	Itapetininga-SP	2005
EH-1441	<i>S. lycopersicum</i>	Itapetininga-SP	2005
EH-1467	<i>S. tuberosum</i>	Itapetininga-SP	2005
EH-1469	<i>S. tuberosum</i>	Itapetininga-SP	2005
EH-1489	<i>N. tabacum</i>	Tijucas-SC	2006
EH-1495	<i>N. tabacum</i>	Tijucas-SC	2006
EH-1505	<i>S. melongena</i>	Brasília-DF	2006
EH-1509	<i>S. melongena</i>	Gama-DF	2006
EH-1511	<i>S. lycopersicum</i>	Gama-DF	2006
EH-1534	<i>S. lycopersicum</i>	Marechal Floriano-ES	2006
EH-1548	<i>S. tuberosum</i>	Gama-DF	2006
EH-1549	<i>S. tuberosum</i>	Gama-DF	2006
EH-1559	<i>S. tuberosum</i>	Ibicoara-BA	2007
EH-1642	<i>S. lycocarpum</i>	Ibicoara-BA	2006
EH-1643	<i>S. lycocarpum</i>	Ibicoara-BA	2006
EH-1654	<i>N. physalodes</i>	Gama-DF	2006

EH-1655	<i>N. physalodes</i>	Gama-DF	2006
EH-1672	<i>S. lycopersicum</i>	Araguari-MG	2006
EH-1674	<i>S. lycopersicum</i>	Taquari-RS	2006
EH-1675	<i>S. lycopersicum</i>	Taquari-RS	2006
EH-1687	<i>S. lycocarpum</i>	Gama-DF	2006
EH-1707	<i>S. lycopersicum</i>	Guaraí-TO	2007
EH-1712	<i>S. tuberosum</i>	Niquelândia-GO	2007
EH-1738	<i>D. stramonium</i>	Gama-DF	2008
EH-1792	<i>S. lycopersicum</i>	Nova Friburgo-RJ	2008
EH-1799	<i>S. lycopersicum</i>	Brasília-DF	2009
EH-1800	<i>S. tuberosum</i>	Itapetininga-SP	2009
EH-1801	<i>S. tuberosum</i>	Itapetininga-SP	2009
EH-1802	<i>S. tuberosum</i>	Itapetininga-SP	2009
EH-1806	<i>S. melongena</i>	Camocim de São Félix-PE	2009
EH-1821	<i>S. lycopersicum</i>	Ubajara-CE	2009
EH-1822	<i>S. lycopersicum</i>	Ubajara-CE	2009
EH-1823	<i>S. lycopersicum</i>	Ubajara-CE	2009
EH-1834	<i>S. lycopersicum</i>	Guaíra-SP	2009
EH-1835	<i>S. lycopersicum</i>	Cafelândia-SP	2009
EH-1836	<i>S. lycopersicum</i>	Cafelândia-SP	2009
EH-1846	<i>S. lycopersicum</i>	Planaltina-DF	2009
EH-1888	<i>S. lycopersicum</i>	Rio Verde-GO	2009
EH-1892	<i>S. lycopersicum</i>	Piracanjuba-GO	2009
EH-1903	<i>S. lycopersicum</i>	Gama-DF	2009
EH-1904	<i>S. lycopersicum</i>	Gama-DF	2009
EH-1911	<i>S. lycopersicum</i>	Planaltina-DF	2009
EH-1912	<i>S. lycopersicum</i>	Planaltina-DF	2009
EH-1925	<i>D. stramonium</i>	Gama-DF	2009
EH-1926	<i>D. stramonium</i>	Gama-DF	2009
EH-1952	<i>S. commersonii</i>	Lebon Régis-SC	2010
EH-1971	<i>S. tuberosum</i>	Bom Jesus-RS	2010
EH-1975	<i>S. lycopersicum</i>	Planaltina-DF	2010
EH-1976	<i>S. lycopersicum</i>	Planaltina-DF	2010
EH-1996	<i>S. lycopersicum</i>	Morrinhos-GO	2010
EH-2061	<i>S. lycopersicum</i>	Planaltina-GO	2011
EH-2062	<i>S. lycopersicum</i>	Planaltina-GO	2011
EH-2112	<i>S. lycopersicum</i>	Brasília de Minas-MG	2013
EH-2113	<i>S. lycopersicum</i>	Brasília-DF	2013
EH-2115	<i>S. lycopersicum</i>	Taquara-DF	2013
EH-2116	<i>N. physalodes</i>	Gama-DF	2013
EH-2117	<i>N. physalodes</i>	Gama-DF	2013
EH-2119	<i>S. lycopersicum</i>	Gama-DF	2013

EH-2120	<i>S. lycopersicum</i>	Capão Bonito-SP	2013
EH-2121	<i>S. lycopersicum</i>	Gama-DF	2013

^aEH: Embrapa Hortaliças.

^bCodes for the Brazilian regions: BA = Bahia; CE = Ceará; DF = Distrito Federal; ES = Espírito Santo; GO = Goiás; MG = Minas Gerais; PE = Pernambuco; PR = Paraná; RJ = Rio de Janeiro; RS = Rio Grande do Sul; SC = Santa Catarina; SP = São Paulo; TO = Tocantins.

Table 2 – Collection of Brazilian and worldwide isolates of *Alternaria* species (with their respective hosts and geographical locations) employed in the present diversity analysis with their corresponding glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*), calmodulin, the *A. alternata* allergen protein-coding (*Alt a1*) gene sequences.

<i>Alternaria</i> species and isolate code	Genomic region / GenBank accession number			Host	Place
	<i>Alt a1</i>	<i>GAPDH</i>	Calmodulin		
<i>A. mimicula</i> BMP 0324	GQ180094	GQ180078	---	---	---
<i>A. mimicula</i> EGS 01.056	---	---	JQ646176	---	---
<i>A. tomato</i> CBS 114.35	KP123992	KP124295	JQ646195	<i>Solanum lycopersicum</i>	---
<i>A. tomato</i> CBS 103.30	KP123991	KP124294	---	<i>Solanum lycopersicum</i>	---
<i>A. alternata</i> CBS 620.83	KP123868	KP124171	---	<i>Nicotiana tabacum</i>	USA
<i>A. alternata</i> CBS 639.97	KP123876	KP124182	---	<i>Helianthus annuus</i>	Greece
<i>A. alternata</i> CBS 118814	KP123906	---	---	<i>Solanum lycopersicum</i>	USA
<i>A. alternata</i> CBS 118815	KP123907	---	---	<i>Solanum lycopersicum</i>	USA
<i>A. alternata/tenuissima</i> BMP 264	KY626355	---	KY626613	---	---
<i>A. alternata</i> CBS 966.95	KP123873	---	---	<i>Solanum lycopersicum</i>	India
<i>A. alternata</i> CBS 113013	KP123889	KP124195	---	<i>Malus domestica</i>	South Africa
<i>A. alternata</i> CBS 116749	KP123901	---	---	---	Netherlands
<i>A. alternata/tenuissima</i> BMP 252	KY626346	---	KY626588	<i>Solanum lycopersicum</i>	---
<i>A. alternata/tenuissima</i> BMP 254	KY626347	---	KY626589	<i>Solanum lycopersicum</i>	---
<i>A. alternata/tenuissima</i> BMP 255	KY626348	---	KY626590	<i>Solanum lycopersicum</i>	---
<i>A. alternata/tenuissima</i> BMP 258	KY626351	---	KY626611	<i>Solanum lycopersicum</i>	---
<i>A. alternata/tenuissima</i> BMP 260	KY626353	---	KY626612	<i>Solanum lycopersicum</i>	---
<i>A. alternata/tenuissima</i> BMP 261	KY626354	---	KY626591	<i>Solanum lycopersicum</i>	---
<i>A. alternata/tenuissima</i> BMP 276	KY626356	---	KY626592	<i>Solanum lycopersicum</i>	---
<i>A. aff. arborescens</i> CBS 105.24	KP123941	KP124245	---	<i>Solanum tuberosum</i>	---
<i>A. aff. arborescens</i> CBS 109730	KP123946	KP124251	---	<i>Solanum lycopersicum</i>	USA
<i>A. arborescens</i> CBS 102605	AY563303	AY278810	---	---	---
<i>A. arborescens</i> BMP 240	KY626345	---	KY626632	<i>Solanum lycopersicum</i>	---
<i>A. arborescens</i> BMP 257	KY626350	---	KY626634	<i>Solanum lycopersicum</i>	---
<i>A. arborescens</i> BMP 3088	KY626368	---	KY626646	<i>Solanum tuberosum</i>	---
<i>A. arborescens</i> BMP 239	KY626344	---	KY626631	<i>Solanum lycopersicum</i>	---
<i>A. arborescens</i> BMP 256	KY626349	---	KY626633	<i>Solanum lycopersicum</i>	---

<i>A. arborescens</i> BMP 259	KY626352	---	KY626635	<i>Solanum lycopersicum</i>	---
<i>A. arborescens</i> BMP 3105	KY626370	---	KY626647	<i>Daucus carota</i>	---
<i>A. logipes</i> CBS 113.35	KP123986	KP124289	---	<i>Nicotiana tabacum</i>	---
<i>A. logipes</i> 30.033	AY563304	AY278811	JQ646198	<i>Nicotiana tabacum</i>	---
<i>A. capsici</i> BMP 0180	AY563298	AY562408	JQ646256	---	---
<i>A. crassa</i> BMP 0172	AY563293	AY278804	---	---	---
<i>A. dauci</i> BMP 167	HE796725	HE796762	JQ646241	<i>Daucus carota</i>	USA
<i>A. dauci</i> BA 1491	HE796726	HE796763	---	<i>Daucus carota</i>	New Zealand
<i>A. argyroxiphii</i> EGS 35.122	JQ646434	JQ646350	JQ646260	---	---
<i>A. argyroxiphii</i> PPRI 11848	KJ718633	KJ717965	---	<i>Ipomoea batatas</i>	South Africa
<i>A. cythomandrae</i> CBS 109155	JQ646444	JQ646360	JQ646270	<i>Solanum betaceum</i>	New Zealand
<i>A. solani-nigri</i> CBS 113403	JQ646417	JQ646333	JQ646233	<i>Solanum nigrum</i>	India
<i>A. cretica</i> CBS 109164	JQ646426	JQ646342	JQ646250	---	---
<i>A. cretica</i> EGS 46.188	---	GQ180074	---	---	---
<i>A. subcylindrical</i> EGS 45.113	---	GQ180084	---	---	---
<i>A. subcylindrical</i> CBS 109161	JQ646429	JQ646345	JQ646254	---	---
<i>A. grandis</i> CBS109158	JQ646425	JQ646341	JQ646249	<i>Solanum tuberosum</i>	---
<i>A. grandis</i> CBS 116695	KJ718748	---	---	<i>Solanum tuberosum</i>	USA
<i>A. grandis</i> NB251	---	KR911750	KR911766	<i>Solanum lycopersicum</i>	Algeria
<i>A. grandis</i> EGS 44.106	GQ180093	GQ180077	---	<i>Solanum tuberosum</i>	Brazil
<i>A. grandis</i> NB 248	---	---	KR911763	<i>Solanum lycopersicum</i>	Algeria
<i>A. grandis</i> NB 249	---	---	KR911764	<i>Solanum lycopersicum</i>	Algeria
<i>A. protenta</i> CBS 347.79	KJ718728	KJ718054	---	<i>Solanum lycopersicum</i>	New Zealand
<i>A. protenta</i> CBS 116437	KJ718729	KJ718055	---	<i>Hordeum vulgare</i>	New Zealand
<i>A. solani</i> CBS 106.21	KJ718743	KJ718066	---	---	---
<i>A. solani</i> CBS 111.41	KJ718744	---	---	<i>Solanum aviculare</i>	---
<i>A. solani</i> EGS 44.098	GQ180096	GQ180080	KJ397981	---	Brazil
<i>A. solani</i> EGS 45.020	GQ180097	---	---	<i>Solanum tuberosum</i>	Brazil
<i>A. solani</i> EGS 45.053	GQ180098	---	---	<i>Solanum tuberosum</i>	Brazil
<i>A. solani</i> EGS 46.178	GQ180099	---	---	<i>Solanum tuberosum</i>	Brazil
<i>A. solani</i> MF-P048011	KJ397975	KJ397984	KJ397979	---	Russia
<i>A. solani</i> MF-P0585011	KJ397978	---	KJ397983	---	Russia
<i>A. solani</i> NB 237	---	KR911751	KR911770	<i>Solanum tuberosum</i>	Algeria
<i>A. solani</i> NB 250	---	---	KR911765	<i>Solanum lycopersicum</i>	Algeria
<i>A. solani</i> NB 252	---	---	KR911767	<i>Solanum lycopersicum</i>	Algeria
<i>A. solani</i> NB 266	---	---	KR911769	<i>Solanum lycopersicum</i>	Algeria
<i>A. solani</i> NB 292	---	---	KR911768	<i>Solanum lycopersicum</i>	Algeria
<i>A. solani</i> NB 314	---	---	KT937240	<i>Solanum lycopersicum</i>	Algeria
<i>A. solani</i> NB 315	---	---	KT937241	<i>Solanum lycopersicum</i>	Algeria
<i>A. solani</i> NB 317	---	---	KT937242	<i>Solanum lycopersicum</i>	Algeria
<i>A. solani</i> NB 318	---	---	KT937243	<i>Solanum lycopersicum</i>	Algeria

<i>A. solani</i> NB 320	---	---	KT937244	<i>Solanum lycopersicum</i>	Algeria
<i>A. linariae</i> CBS 109156	JQ646431	JQ646347	JQ646257	---	-
<i>A. linariae</i> EGS 42.156	GQ180101	GQ180085	---	<i>Solanum lycopersicum</i>	Brazil
<i>A. linariae</i> MF-P138061	KJ397976	KJ397985	KJ397980	---	Russia
<i>A. linariae</i> MF-P580171	KJ397977	---	KJ397982	---	Russia
<i>A. linariae</i> NB 211	---	---	KR911771	<i>Solanum lycopersicum</i>	Algeria
<i>A. linariae</i> NB 242	---	KR911762	KR911777	<i>Solanum lycopersicum</i>	Algeria
<i>A. linariae</i> NB 243	---	---	KR911772	<i>Solanum lycopersicum</i>	Algeria
<i>A. linariae</i> NB 264	---	KR911760	KR911776	<i>Solanum lycopersicum</i>	Algeria
<i>A. linariae</i> NB 261	---	KR911761	KR911778	<i>Solanum lycopersicum</i>	Algeria
<i>A. linariae</i> NB 262	---	---	KR911775	<i>Solanum lycopersicum</i>	Algeria
<i>A. linariae</i> NB 272	---	KR911758	KR911774	<i>Solanum lycopersicum</i>	Algeria
<i>A. linariae</i> NB 307	---	---	KR911773	<i>Solanum lycopersicum</i>	Algeria
EH-0568	---	MT968780	---	<i>Solanum lycopersicum</i>	Brazil
EH-0643	MT980730	MT968781	---	<i>Solanum tuberosum</i>	Brazil
EH-0679	MT980731	MT968782	MT951434	<i>Solanum melongena</i>	Brazil
EH-0680	---	MT968783	---	<i>Solanum melongena</i>	Brazil
EH-0694	MT980732	MT968784	---	<i>Solanum tuberosum</i>	Brazil
EH-0742	---	MT968785	---	<i>Solanum lycopersicum</i>	Brazil
EH-0768	MT980733	MT968786	---	<i>Solanum lycopersicum</i>	Brazil
EH-0809	MT980734	MT968787	MT951435	<i>Solanum tuberosum</i>	Brazil
EH-0862	MT980735	MT968788	MT951429	<i>Solanum lycopersicum</i>	Brazil
EH-1120	MT980736	MT968789	---	<i>Solanum lycopersicum</i>	Brazil
EH-1122	---	MT968790	---	<i>Solanum lycopersicum</i>	Brazil
EH-1143	MT980737	MT968791	MT951443	<i>Solanum lycopersicum</i>	Brazil
EH-1168	MT980738	MT968792	---	<i>Capsicum annuum</i>	Brazil
EH-1174	MT980739	MT968793	---	<i>Solanum lycopersicum</i>	Brazil
EH-1176	MT980740	MT968794	---	<i>Solanum lycopersicum</i>	Brazil
EH-1180	MT980741	MT968795	MT951436	<i>Solanum tuberosum</i>	Brazil
EH-1183	MT980742	MT968796	---	<i>Solanum tuberosum</i>	Brazil
EH-1185	MT980743	MT968797	---	<i>Solanum tuberosum</i>	Brazil
EH-1208	MT980744	MT968798	---	<i>Solanum lycopersicum</i>	Brazil
EH-1209	---	MT968799	---	<i>Solanum lycopersicum</i>	Brazil
EH-1210	---	MT968800	---	<i>Solanum lycopersicum</i>	Brazil
EH-1214	MT980745	MT968801	MT951430	<i>Solanum lycopersicum</i>	Brazil
EH-1233	MT980746	MT968802	---	<i>Solanum tuberosum</i>	Brazil
EH-1249	MT980747	MT968803	---	<i>Solanum tuberosum</i>	Brazil
EH-1252	MT980748	MT968804	MT951442	<i>Solanum lycopersicum</i>	Brazil
EH-1258	---	MT968805	---	<i>Solanum lycopersicum</i>	Brazil
EH-1261	MT980749	MT968806	MT951437	<i>Solanum lycopersicum</i>	Brazil
EH-1266	---	MT968807	---	<i>Solanum tuberosum</i>	Brazil

EH-1332	MT980750	MT968808	---	<i>Solanum tuberosum</i>	Brazil
EH-1336	---	MT968809	---	<i>Solanum tuberosum</i>	Brazil
EH-1341	MT980752	MT968810	---	<i>Solanum tuberosum</i>	Brazil
EH-1381	MT980753	MT968811	---	<i>Solanum tuberosum</i>	Brazil
EH-1406	MT980754	MT968812	MT951431	<i>Capsicum annuum</i>	Brazil
EH-1435	MT980755	MT968813	MT951438	<i>Solanum lycopersicum</i>	Brazil
EH-1437	MT980756	MT968814	---	<i>Solanum lycopersicum</i>	Brazil
EH-1467	MT980757	MT968815	---	<i>Solanum tuberosum</i>	Brazil
EH-1489	MT980758	MT968816	---	<i>Nicotiana tabacum</i>	Brazil
EH-1495	MT980759	MT968817	---	<i>Nicotiana tabacum</i>	Brazil
EH-1509	MT980760	MT968818	---	<i>Solanum melongena</i>	Brazil
EH-1534	---	MT968819	---	<i>Solanum lycopersicum</i>	Brazil
EH-1548	MT980761	MT968820	MT951439	<i>Solanum tuberosum</i>	Brazil
EH-1559	MT980762	MT968821	---	<i>Solanum tuberosum</i>	Brazil
EH-1642	MT980763	MT968822	---	<i>Solanum lycocarpum</i>	Brazil
EH-1654	MT980764	MT968823	MT951432	<i>Nicandra physalodes</i>	Brazil
EH-1655	MT980765	MT968824	MT951433	<i>Nicandra physalodes</i>	Brazil
EH-1674	---	MT968825	---	<i>Solanum lycopersicum</i>	Brazil
EH-1675	---	MT968826	---	<i>Solanum lycopersicum</i>	Brazil
EH-1707	---	MT968827	---	<i>Solanum lycopersicum</i>	Brazil
EH-1712	MT980766	MT968828	---	<i>Solanum tuberosum</i>	Brazil
EH-1792	MT980767	MT968829	---	<i>Solanum lycopersicum</i>	Brazil
EH-1802	MT980768	MT968830	---	<i>Solanum tuberosum</i>	Brazil
EH-1806	MT980769	MT968831	---	<i>Solanum melongena</i>	Brazil
EH-1823	MT980770	MT968832	MT951440	<i>Solanum lycopersicum</i>	Brazil
EH-1836	MT980771	MT968833	MT951441	<i>Solanum lycopersicum</i>	Brazil
EH-1892	---	MT968834	---	<i>Solanum lycopersicum</i>	Brazil
EH-1925	---	MT968835	---	<i>Datura stramonium</i>	Brazil
EH-1926	---	MT968836	---	<i>Datura stramonium</i>	Brazil
EH-1952	---	MT968837	---	<i>Solanum commersonii</i>	Brazil
EH-2113	MT980772	MT968838	MT951444	<i>Solanum lycopersicum</i>	Brazil
EH-2115	MT980773	MT968839	---	<i>Solanum lycopersicum</i>	Brazil
EH-2116	---	MT968840	---	<i>Nicandra physalodes</i>	Brazil

Table 3 Pathogenicity of a selected group of Brazilian *Alternaria* isolates obtained from foliar lesions of Solanaceae hosts on cultivated and non-cultivated species from the Solanaceae genera (*Solanum*, *Nicandra*, *Datura*, *Nicotiana* and *Capsicum*).

Isolates/ collection place ^a	Original host ^b	<i>Alternaria</i> species	Inoculated Solanaceae species							
			SL	ST	CA	SM	SAG	DS	PA	NT
EH-1143 / RS	SL	<i>A. dauci</i>	(+) ^c	(-)	(-)	(-)	(-)	(-)	(-)	NE
EH-1210 / PR	SL	<i>A. argyroxiphii</i>	(+)	(-)	NE	NE	NE	NE	NE	NE
EH-1214 / DF	SL	<i>A. argyroxiphii</i>	(+)	(-)	(-)	(-)	(-)	(-)	(+)	NE
EH-1252 / SP	SL	<i>A. crassa</i>	(+)	(+)	(-)	(-)	(-)	(-)	(+)	NE
EH-2113 / DF	SL	<i>Alternaria</i> sp.	(+)	(+)	(-)	(-)	(-)	(+)	(+)	NE
EH-1548 / DF	ST	<i>A. grandis</i>	(+)	(+)	(-)	(-)	(+)	(-)	(-)	NE
EH-0679 / MG	SM	<i>A. grandis</i>	(+)	(+)	(-)	(-)	(+)	(+)	(-)	NE
EH-1642 / BA	SLY	<i>A. linariae</i>	(+)	(+)	(-)	(+)	(+)	(+)	(+)	NE
EH-1654 / DF	NP	<i>A. argyroxiphii</i>	(+)	(-)	(-)	(-)	(-)	(-)	(-)	NE
EH-1738 / DF	DS	<i>A. crassa</i>	(+)	(+)	(-)	(-)	(+)	(+)	(-)	NE
EH-1823 / CE	SL	<i>A. linariae</i>	(+)	(+)	NE	NE	NE	NE	NE	NE
EH-1489 / SC	NT	<i>A. longipes</i>	NE	NE	NE	NE	NE	NE	NE	(+)
EH-1495 / SC	NT	<i>A. longipes</i>	NE	NE	NE	NE	NE	NE	NE	(+)

^aCodes for the Brazilian States: BA = Bahia; CE = Ceará; DF = Federal District; MG = Minas Gerais; RS = Rio Grande do Sul; SC= Santa Catarina; SP = São Paulo.

^bCodes for the original host and inoculated Solanaceae species: SL = tomato (*Solanum lycopersicum*); ST = potato (*S. tuberosum*); SLY= wolf-fruit (*S. lycocarpum*); CA = sweet pepper (*Capsicum annuum*); SM = eggplant (*S. melongena*); SAG = scarlet eggplant (*S. aethiopicum* var. *gilo*); DS = jimson weed (*Datura stramonium*); PA = *Physalis angulata*; NP = *Nicandra physalodes*; NT= tobacco (*Nicotiana tabacum*)

^cPlant species reaction: (+) = presence of typical early-blight symptoms; (-) = absence of symptoms; and (NE) = not evaluated.

Table 4 Morphological characterization of 17 *Alternaria* isolates obtained from foliar lesions of cultivated, weed, and native Solanaceae hosts in Brazil.

Host / Isolate	Species	Rostrum number (%)	Conidial body		Rostrum length	Septa	
		1/2/3	Length (µm)	Width (µm)	Length 1/2/3 (µm)	Trans.	Long.
<i>S. lycopersicum</i>							
EGS 42-156	<i>A. linariae</i>	11/65/24	70–99	16 –20	147–216/144–184/97–172	7–12	1–4
AS440	<i>A. cretica</i>	62/34/4	70–100	12–16	133–197/126–199/121–147	7–12	0–3
	<i>A. dauci</i>		40-110	10-25	-	5-9	-
	<i>A. crassa</i>		47-168	15-32	100-439	6-12	1-8
	<i>A. argyroxiphii</i>		70-100	16-20	100-200	9-12	1-2
EH-1143			33.32–48.94	8.67–15.70	3.72–14.84	4–6	0–4
EH-1210		72/28	60.93–98.74	12.67–25.92	178.18–282.87	6–10	0–5
EH-1214			52.47–101.57	18.04–31.64	94.57–236.54	5–10	2–8
EH-1252			59.33–106.79	18.91–34.73	8.50–77.99	5–10	2–8
EH-1261		62/34/4	70.65–125.44	11.99–29.45	97.18–248.24/111.58– 235.64/138.09–194.87	6–12	0–5
EH-1435		84/16	62.33–126.40	11.44–29.25	64.83–210.46/70.05– 164.32	6–11	0–6
EH-1823		60/38/2	69.15–97.85	14.13–19.23	124.23–208.43/95.46– 211.12/145.65–159.87	6–11	1–6
EH-2113			75.47–111.29	19.18–37.79	22.22–72.84	7–12	3–9
<i>S. tuberosum</i> , <i>S. commersonii</i>							
EGS 44-098	<i>A. solani</i>		85–100	18 –22	83–110	8–12	1–3
AS216	<i>A. grandis</i>		104–161	14 –17	156–206	11–14	0–3
EH-0809			91.66–140.87	13.03 –20.68	81,35–200,98	8–12	0–4
EH-1180			116.38–247.63	15.95 –24.18	95,91–221,49	9–18	0–5
EH-1548			110.54–163.38	18.63 –29.63	87,99–182,55	9–16	0–4
EH-1952			68.87–103.40	11.46 –18.53	69,44–260,09	7–11	0–4
Eggplant, native flora, and weed hosts ^a							
EH-0679-Sm		46/50/4	89.44–144.57	16.61–26.75	43.08–183.39/48.45–181.88	8–13	2–6
EH-1642-Sly		34/56/8/2	60.48–93.98	17.77–23.67	124.01–191.98/113.61– 192.14/44.82–161.18/77.49– 214.58	5–8	1–5
EH-1654-Np		98/2	55.53–80.05	14.38–21.82	23.97–154.97/51.46–110.50	6–11	2–7
EH-1738-Ds			82.09–121.93	16.28–28.94	67.61–188.77	7–11	0–4
EH-2116-Np		96/4	69.63–101.07	16.28–26.78	81.10–160.84	6–9	0–4

^aDs = *Datura stramonium*, Np = *Nicandra physalodes*, Sly = *Solanum lycocarpum* (wolf-fruit), Sm = *S. melongena* (eggplant).

Figure 1 Geographic distribution of *Alternaria* isolates associated early blight (EB)–like foliar lesions in different species of Solanaceae in Brazil. The acronyms of the Brazilian geographic regions are: Bahia (BA), Ceará (CE), Pernambuco (PE), the Federal District (DF), Espírito Santo (ES), Goiás (GO), Minas Gerais (MG), Rio de Janeiro (RJ), São Paulo (SP), Rio Grande do Sul (RS), Santa Catarina (SC), Paraná (PR), and Tocantins (TO).

Figure 2 Phylogenetic analysis by Bayesian, Maximum Parsimony and Maximum Likelihood inferences of *Alternaria* isolates from Solanaceae hosts in Brazil representing distinct haplotypes for the *Alternaria alternata* allergenic protein–coding gene (*Alt a1*). Dashes in the nodes are indicating values lower than 0.80 for posterior probability and lower than 60% for Values of Maximum Parsimony and Maximum Likelihood.

Figure 3 Phylogenetic analysis by Bayesian, Maximum Parsimony and Maximum Likelihood inferences of representative *Alternaria* isolates from Solanaceae hosts in Brazil representing distinct haplotypes for the glyceraldehyde–3–phosphate dehydrogenase (*GAPDH*) gene. Dashes in the nodes are indicating values lower than 0.80 for posterior probability and lower than 60% for Values of Maximum Parsimony and Maximum Likelihood.

Figure 4. Phylogenetic analysis by Bayesian, Maximum Parsimony and Maximum Likelihood inferences of representative *Alternaria* isolates from Solanaceae hosts in Brazil representing distinct haplotypes for the calmodulin gene region. Dashes in the nodes are indicating values lower than 0.80 for posterior probability and lower than 60% for Values of Maximum Parsimony and Maximum Likelihood.

Figure S1. Phylogenetic analysis by Bayesian, Maximum Parsimony and Maximum Likelihood inferences from *Alternaria alternata* allergenic protein-coding gene (*Alt a1*) of 120 *Alternaria* isolates from Solanaceae hosts in Brazil. Values represented by a dash were lower than 0.80 for posterior probability and lower than 60% for Values of Maximum Parsimony and Maximum Likelihood.

Fig 1

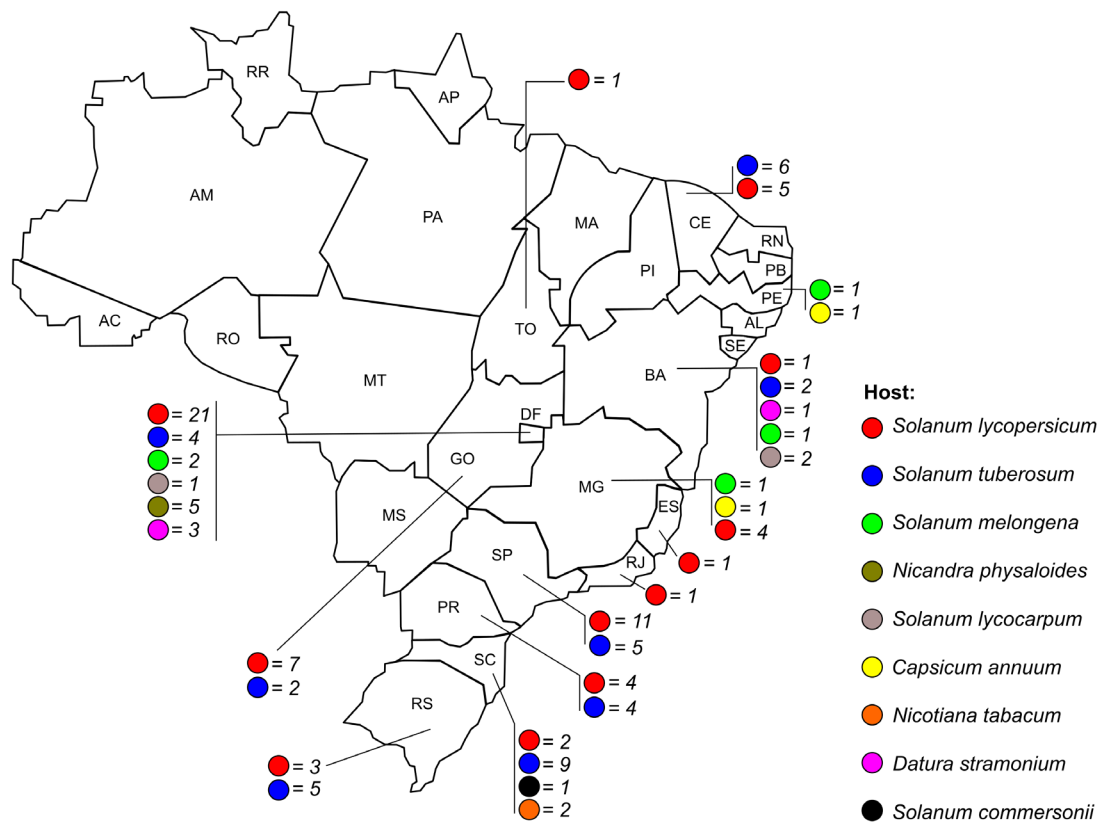


Fig 2.

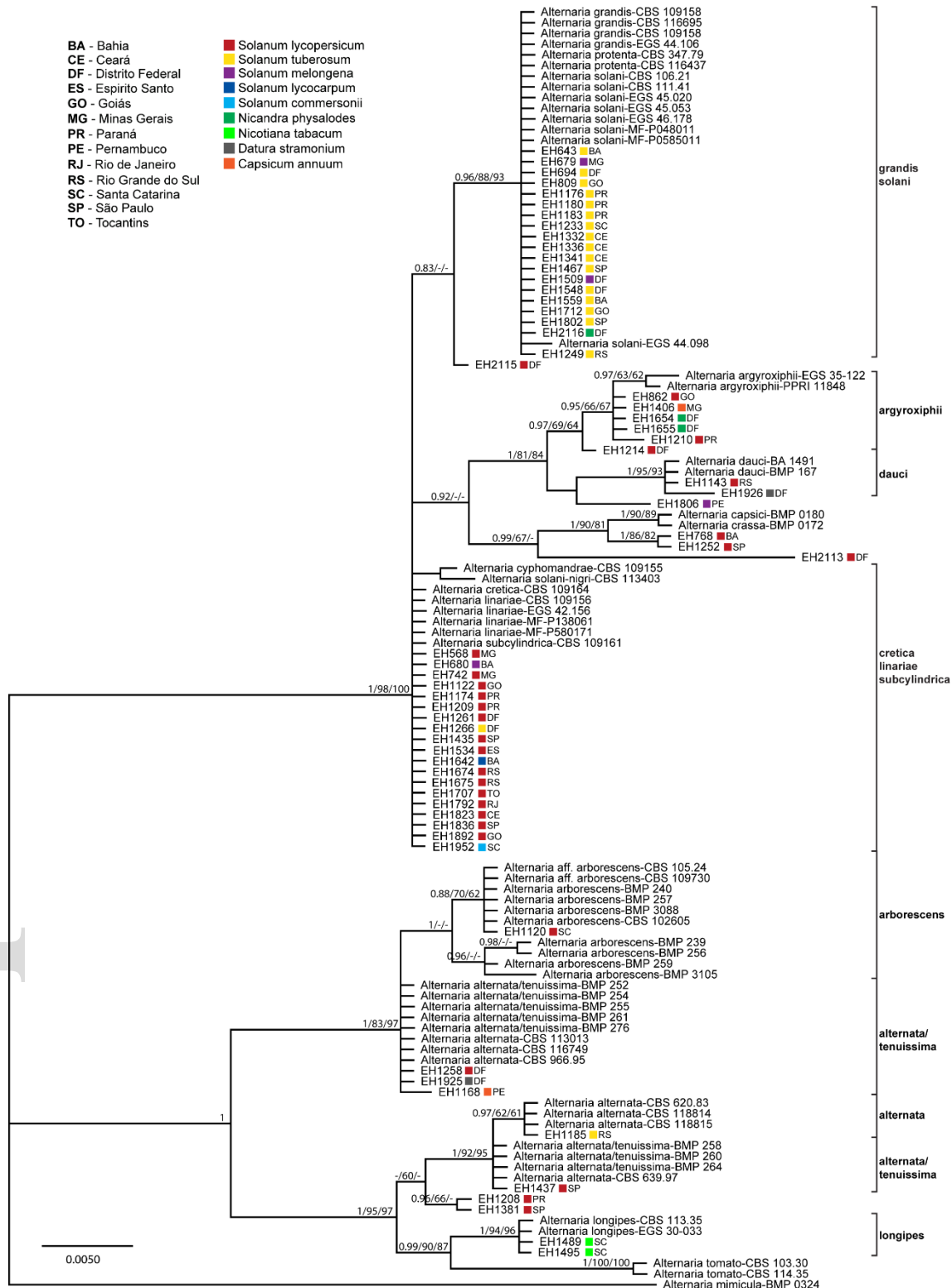


Fig 3.

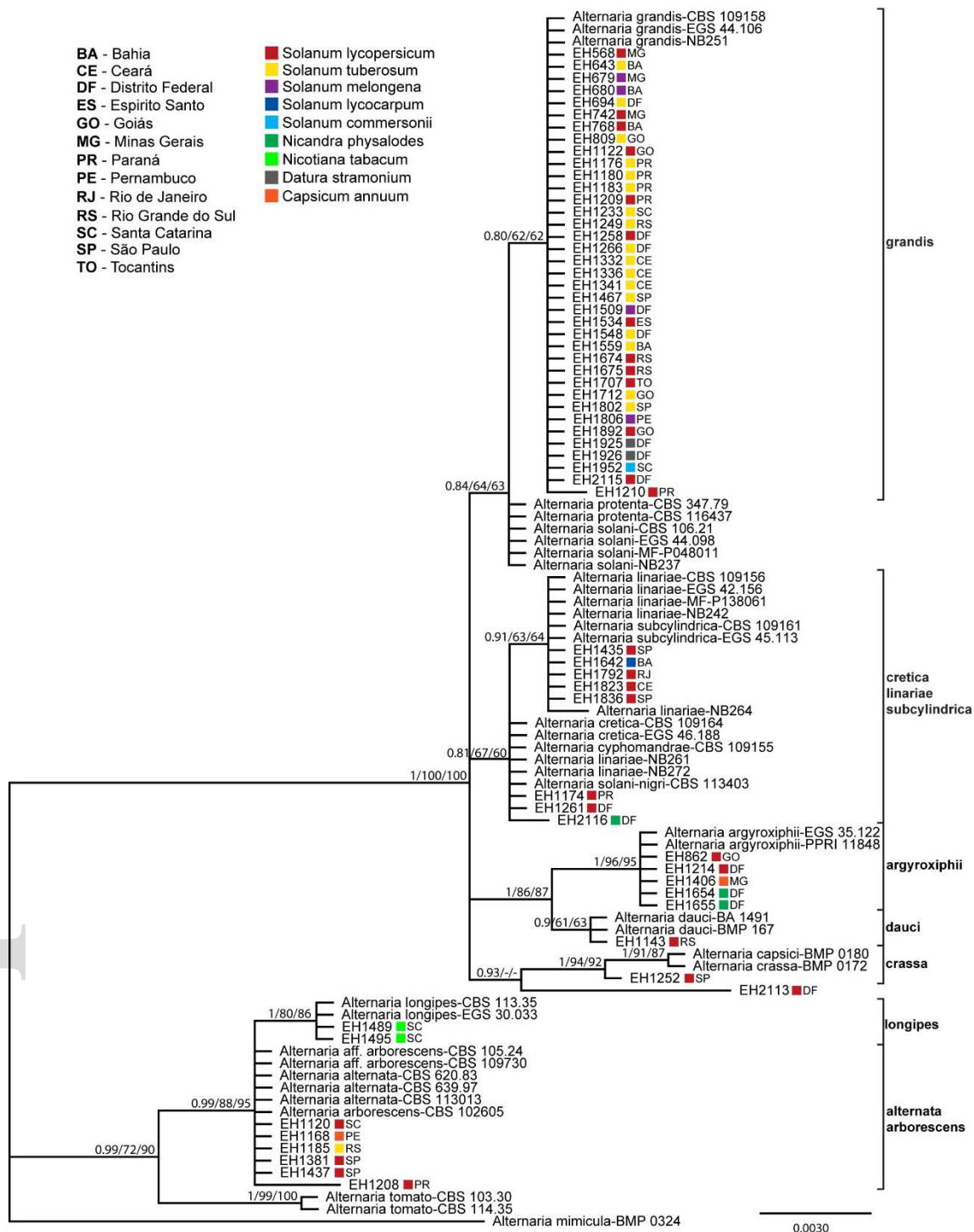


Fig 4.



