First report of *Lasiodiplodia theobromae* causing stem rot disease of begonia (*Begonia x elatior* hort.) in Brazil

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Abstract *Lasiodiplodia theobromae* was found causing stem rot on commercial production of *Begonia x elatior* in São Paulo, Brazil. Illustrations, morphological and molecular description are provided. Based on the morphology, this fungus was recognized as *L. theobromae*. However, *L. theobromae* has high similarity with other *Lasiodiplodia* species, some of which are not possible to be separated by morphological characters. Molecular identification of the fungus isolated from the infected tissues was conducted. The strain from begonia clustered with other isolates of *L. theobromae*. This is the first report of the occurrence of *L. theobromae* on *B. elatior*.

Keywords Begonia · Stem rot · *Lasiodiplodia theobromae*

Begonia is a widely cultivated and appreciated genus of ornamental plants because of their versatility in foliar and

flower-colors. The major disease problems of begonias are *Botrytis* blight, powdery mildew, bacterial blight, stem rot and root rot caused by *Pythium* sp. and *Fusarium* sp. (Daughtrey et al. 1995).

The symptom of stem rot and root rot are usually observed in commercial production of begonias, the root system becomes small and discolored (brown), stems become water-soaked, discolored and collapse. The etiology of this has been most of the time attributed to *Fusarium* spp. (Schroers et al. 2004). However, some diseased plants from Brazil showed symptoms of stem rot with presence of black erumpent pycnidia.

Producers of begonia of the state of São Paulo suffered heavy losses with stem rot in 2009–2010. The symptoms observed were necrotic lesion of stem (Fig. 1c) and rotting of stems (Fig. 1a), followed by death of the plants in most severe cases. Small to medium-sized black hard erumpent pycnidia were seen on affected stem. Infected stems exhibited internal discoloration and browning of vascular regions. To determine the etiology of these symptoms, diseased plants were collected during January to April of 2010. The fungus presents mycelium initially white changing afterwards to dark and finally black. Slides with diseased tissue were mounted in lactophenol and examined microscopically. In sections, it was possible to observe black pycnidia, isolated, erumpent, pyriform with apical ostiole, confluent, with dimensions of 138–178×136–204 µm and stromatic, composed of several layers dark, thick-walled (Fig. 1d, e and f). The conidia were hyaline and unicellular when immature (Fig. 1g, h and i), and then became dark and 2-celled, ovoid to elongate, with perpendicular striations to the septum with dimensions of 21.75–27.77×11.27–14.66 µm (Fig. 1j, k and l).

Based on the morphology, this fungus was recognized as *Lasiodiplodia theobromae* (Pat.) Griffon & Maubl. (syn.

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Fig. 1 Stem root of *Lasiodiplodia theobromae* on *Begoniaxelator* **a** Inoculated plant. **b** Detail of the portion of the inoculated portion showing stem rot. **c** Necrotic lesion and pycnidia. **d**, **e** and **f** Pycnidia (pyriform and rostrum) on transversal sections of the stem showing the

relationship of parasitism of the fungus. **g** and **h** Conidia still attached to the conidiogenous cells (arrow). **i** Immature conidia (hyaline and unicellular). **j** Conidia early maturation. **k** and **l** Mature conidia, brown globose, ovoid with transverse septum with longitudinal striations

Botryodiplodia theobromae (Pat.), according to the descriptions of Punithalingam (1976), anamorphic form of *Botryosphaeria rhodina* (Berk. & M.A.Curtis) Arx (von Arx 1974). The culture of the fungal strain and infected tissues were deposited in the Mycological Reference Collection of the University of Brasília, collection number UB 21879.

Pathogenicity tests were performed with mycelial plugs (1 cm diameter) containing reproductive structures which were taken from a two-weeks-old colony growing on PDA and placed on wounded stem of 6 weeks-old-healthy plants of *B. elatior*. The inoculated plants were maintained in a moist chamber (70 % relative humidity) at 25 ± 2 °C with a 12-h photoperiod.

Necrotic symptoms with black pycnidia (Fig. 1c), similar to those described were detected after 2 weeks of the inoculation on stem. The control stem, on which only PDA plugs were placed, remained healthy. The fungus was reisolated from inoculated plants, completing Koch's postulates.

Molecular identification of the fungus isolated from the infected tissues was conducted. Genomic DNA was extracted from the fungal mycelium according to O'Donnell et al. (1998). The ITS region and elongation factor 1- α were amplified using primers ITS 4 and ITS 5 (White et al. 1990), and EF728F and EF986R (Carbone and Kohn 1999), respectively. The polymerase chain reaction consisted of 1 μ L of gDNA, 50 μ M

Table 1 *Lasiodiplodia* spp. compared in the phylogenetic study

Original Isolate Code	Identity	GenBank access code	
		ITS	EF1- α^1
Begonia ²	<i>L. theobromae</i>	JN997383	JN997382
IRAN1518C	<i>L. pseudotheobromae</i>	GU973874.1	GU973866.1
CJA36	<i>L. pseudotheobromae</i>	GU973875.1	GU973867.1
CBS374.54	<i>L. pseudotheobromae</i>	EF622080.1	EF622059.1
CBS116459	<i>L. pseudotheobromae</i>	EF622059.1	EF622057.1
128/62/8	<i>L. parva</i>	GQ896383.1	GU046786.1
CBS494.78	<i>L. parva</i>	EF622084.1	EF622064.1
CBS456.78	<i>L. parva</i>	EF622083.1	EF622063.1
WWF37	<i>L. theobromae</i>	GQ845099.1	GQ850471.1
CJA199	<i>L. theobromae</i>	GU973872.1	GU973864.1
UF05161	<i>L. theobromae</i>	GQ845096.1	GQ850468.1
CBS115812	<i>L. gonubiensis</i>	DQ458892.1	DQ458877.1

¹ Elongation factor 1-alpha. ² Strain from begonia analyzed in this study

of each deoxynucleotide triphosphate (dNTP), 1.5 mM MgCl₂, 1 × PCR, 0.5 μM of each primer and 0.5 U of Taq DNA polymerase (Invitrogen, Carlsbad, CA, US) in a final volume of 50 μL. The same PCR conditions were used for two genes. The samples were denatured at 94 °C for 5 min, followed by 35 amplification cycles (94 °C for 30 s, 58 °C for 30 s, 72 °C for 1 min) and a final extension (72 °C for 7 min). The amplification products (4 μL) analyzed by electrophoresis on 1.5 % agarose gel using TAE buffer (40 mmol/L TRIS-acetate, 1 mmol/L ethylenediaminetetraacetic acid, pH 8.0) showed fragments with size of 634 and 350 bp, for ITS region and elongation factor 1-alpha, respectively. Nucleotide sequences were determined on

both PCR amplification products at the MacroGen sequencing facility (MacroGen Inc., Seoul, Korea). The obtained nucleotide sequences were compared with sequences deposited in the GenBank database (<http://www.ncbi.nlm.nih.gov/>) using the program BlastN. Both ITS region and elongation factor 1-alpha most closely matched *L. theobromae*, showing greater than 98 % and 97 % of similarity to the sequences from authentic isolates available in GenBank (Abdollahzadeh et al. 2010). However, *L. theobromae* has high similarity with other *Lasiodiplodia* species, some of which are not possible to be separated by morphological characters (Abdollahzadeh et al. 2010).

A phylogenetic analysis based on ITS and elongation factor 1-alpha sequences of *L. theobromae* and other species of *Lasiodiplodia* obtained from GenBank (Table 1) and the begonia isolate was carried out. Nucleotide sequences were analyzed using ME GA5 software for neighbor-joining analyses, using Kimura-2-parameter nucleotide substitution model. Bootstrap values were obtained from 1,000 bootstrap replicates. The strain from begonia clustered with other isolates of *L. theobromae* with high bootstrap support (Fig. 2).

Lasiodiplodia theobromae has a broad host range, a world-wide distribution and is responsible for different diseases in different hosts (Cilliers et al. 1993). This pathogen can cause rot in roots, stem, branches, dieback and post-harvest diseases (Rodrigues 2003; Pereira et al. 2009). This is a well-known pathogen commonly associated with root, collar and stem rot, dieback and post-harvest diseases, but not previously reported as the cause of begonia stem rot.

This is the first report of *L. theobromae* causing stem rot on *B. elatior*. The discovery of the association between the pathogen and stem rot in begonias is of great importance because it allows the development of appropriate and effective strategies for disease management.

Fig. 2 The genetic distance tree based on the concatenated nucleotide sequences of ITS and elongation factor 1-alpha from begonia strain and sequences from *Lasiodiplodia theobromae* and other species of this genus obtained on Genbank. Bootstrap values after 1,000 replicates are expressed as percentages. *L. gonubiensis* was included as an outgroup

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