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Rhizoctonia solani AG4 causes lentil damping-off in Brazil

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Abstract
Rhizoctonia solani is recorded here, for the first time, as a lentil pathogen in Brazil. It was isolated from lentil (*Lens culinaris*) affected by root rot and damping-off. The identity of the fungus was elucidated through a combination of morphological and molecular approaches. Koch's postulates were fulfilled and pathogenicity was confirmed.

Keywords Fungal pathogen · Occurrence · Pulse disease · Root rot

Lentil (*Lens culinaris*) is a key crop for food security (Yadav et al. 2007). Giordano et al. (1988) recognized good potential for lentils to become a more broadly cultivated crop in Brazil. Nevertheless, the crop is usually regarded as "not suited for the hot wet tropics" (Purseglove 1968) and there is a lack of information based on local research on this crop in Brazil, including on plant diseases. In March of 2016, lentils grown in demonstration plots in the campus of the Universidade Federal de Viçosa (state of Minas Gerais, Brazil) were attacked by root rot and damping-off. The combination of high temperatures and wet conditions probably favored disease severity. All plants grown on these plots died before reaching maturity (Fig. 1a, b). Samples were collected, taken to the laboratory for analysis, and a mycelial mat was regularly found in association with the necrotic tissues. A representative sample of necrotic plants was dried in a plant press and deposited in the local herbarium at the Universidade Federal de Viçosa (Accession No VIC 44112).

Homogeneous pure cultures were obtained either by transferring clean hyphal fragments to potato dextrose-agar (PDA) plates with a sterile fine pointed needle or by plating selected surface chlorox-disinfected diseased fragments of roots or stems onto PDA plates. Pure culture colonies were obtained and grown on PDA at 25 °C under a 12 h light regime for 7 days for observation of culture morphology. One representative isolate was deposited in the local culture collection (Accession No COAD 2089).

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Slides were mounted in lactoglycerol. The fungus had the following morphology: Hyphae cylindrical, becoming monilioid with age, 2.5 to 8 μm diam, often branching at a 90 ° angle and presenting a septum next to the branching point of branches, walls hyaline becoming pale brown in older parts of colonies (Fig. 1c); Sclerotia irregularly shaped, grayish sepia. Mycelium was stained with SYBR® Green (Sigma-Aldrich) and observed with a Olympus BX 50 fluorescence microscope and cells were found to be multinucleate (Fig. 1d). This morphology was found to be equivalent to that described by Parmeter and Whitney (1970) for *Rhizoctonia solani.*

To confirm the morphology based identification, genomic DNA of COAD 2089 was extracted using the Wizard Genomic DNA Purification Kit (Promega) by following the manufacturer's instructions. The internal transcribed spacer (ITS) region of ribosomal DNA was PCR amplified with the universal primers ITS4 and ITS5 (White et al. 1990) and sequenced by Macrogen (Korea). The resulting sequence was deposited in GenBank (accession no. MH259594). This sequence had 99% identity with an isolate of *R. solani* AG4-HG-I (Accession No. HG934415).

A pathogenicity test was performed using sixteen 30-day-old healthy lentil plants grown in 2-l pots. These were inoculated by placing one 0.5 cm diam disc taken from 7-day-old cultures grown on PDA next to the base of each of the stems. Eight healthy potted plants belonging to the same group were treated similarly to the inoculated plants but only non-colonized PDA disks were placed next to their stem base. These plants were then covered with a moistened plastic bag for 24 h and maintained in a greenhouse at ca. 28° C and irrigated twice a day. Damping-off symptoms appeared four days after inoculation on all

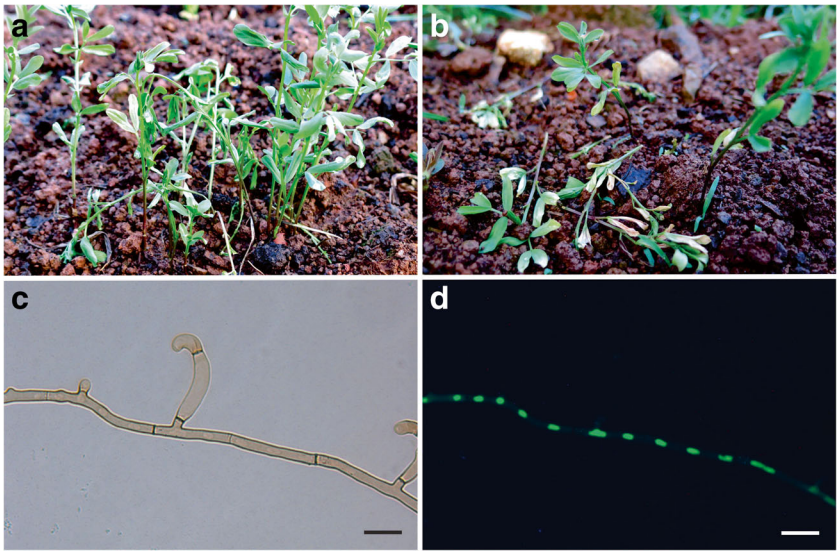

Fig. 1 *Rhizoctonia solani* on *Lens culinaris*. **a** Group of lentil seedlings beginning to show damping-off symptoms. **b** Advanced disease stage with most plantlets fallen after having the stem base girdled. **c** Hypha of *R. solani* (COAD 2089) with typical right-angled branching and constriction at the base of the hyphal branches. **d** SYBR green-stained *R. solani* hypha (same as in **c**) showing multiple fluorescing nuclei within a single cell

inoculated plants. This was followed, two days later, by abundant formation of mycelium. Controls remained healthy. Typical *R. solani* colonies were obtained upon isolations from diseased test-plants.

Although *R. solani* is known to be a broad spectrum pathogen attacking many crops in Brazil, the sole previous record of *R. solani* on lentils in Brazil deals with its detection on seeds (Madeira et al. 1988) and does not include any information on its pathogenic status. Although Taylor et al. (2007) listed collar rot, caused by *R. solani* as a minor disease of the crop, Giordano et al. (1988) considered *R. solani* as causing the worst disease problems for lentil plantations in mid-western Brazil. Nevertheless, this publication did not provide complete details on the taxonomy of the fungus or its pathogenicity. To our knowledge this is the first time *R. solani* has been fully reported and characterized as a lentil pathogen in Brazil.

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