First report of *Rhizoctonia solani* AG1- IA infecting yard-long bean

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Abstract

During March 2016, leaf blight, followed by death, was observed on yard-long bean plants (*Vigna unguiculata* subsp. *sesquipedalis*) in Brazil. Based on morphological characteristics and phylogenetic analysis, the pathogen was identified as *Rhizoctonia solani* AG-1 IA. Koch's postulates were fulfilled. All accessions studied were classified as susceptible. This is the first report of *Rhizoctonia solani* AG1-IA infecting yard-long bean.

Keywords *Vigna unguiculata* subsp. *sesquipedalis* · *Thanatephorus cucumeris* · Leaf blight · Web blight · Anastomosis group

Yard-long bean (*Vigna unguiculata* ssp. *sesquipedalis*), characterized by long and thin pods, is a distinctive subspecies of cowpea. This legume, whose immature pods are an important source of dietary protein, is extensively cultivated by family growers in the state of Pará, Amazon region of Brazil (Cardoso and Barreto 1997). During a period of high rainfall in the region (March, 2016), leaf blight was observed on all yard-long bean plants cv. De Metro planted in a 400 m² experimental plot at Embrapa Amazônia Oriental in Belém, Pará.

White mycelium and microsclerotia were constantly associate with necrotic tissue (Fig. 1A), which eventually led to plant mortality (Fig. 1B). A microscopic examination of necrotic tissue revealed the typical morphological characteristics of *Rhizoctonia*: colorless septate hyphae, a 90° branching pattern, and the constriction of the hyphae near their point of origin (Sneh et al. 1994). Leaf samples with

mycelium were disinfested in 70% alcohol for 30 s and 2% sodium hypochlorite for 30 s. Samples were then washed in distilled water, and plated onto potato dextrose agar (PDA). The Petri dish was maintained at 25° C in the dark. After 10 days, a white to brown culture developed and the formation of microsclerotia was observed (Fig. 1C).

The number of nuclei was determined by staining the hyphal cells with an alkaline safranin solution (0.5% of safranin, 10 mL of KOH 3%, 5 mL of glycerin and 79 mL of distilled water) according to Sneh et al. (1994). The hyphae were examined under an optical microscope (Leica DM 5000), and the number per cell was counted. The isolate had multinucleate hyphal cells; therefore, the anastomosis group (AG) was determined by pairing the isolate with tester strains from the multinucleate *Rhizoctonia solani* group according to Ceresini et al. (1996).

Tester isolates were obtained from the Culture Collection of Microorganisms of Environmental and Agricultural Importance (CMAA) at Embrapa Meio Ambiente, Jaguariúna, São Paulo, Brazil, and grown on PDA in the dark at 25 °C for 48 h (Siviero et al. 2019). After this period, a disc of mycelia from an AG tester was transferred to the extremity of a sterilised microscope slide containing a thin layer of 2% agar-water. On the opposite extremity, a disc of mycelia from the *Rhizoctonia* isolate of yard-long bean was deposited. Each pairing combination (AG tester and the *Rhizoctonia* isolate) was maintained inside petri dishes at 25 °C in the dark. Mycelial growth was observed under an optical microscope (Leica DM 5000) several times a day until hyphal contact.

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Fig. 1 (A) Symptoms of leaf blight associated with white mycelium and microsclerotia on yard-long bean (*Vigna unguiculata* subsp. *sesquipedalis*), (B) dead plants in experimental field, (C) Colony of *Rhizoctonia solani* AG1-IA CMAA 1640, (D) Leaf blight symptoms on inoculated yard-long bean caused by *R. solani* AG1-IA CMAA 1640

The classification of the anastomosis reaction was observed at the point of contact, using the method of Parmeter et al. (1969). The *Rhizoctonia* isolate sampled from yard-long bean anastomosed with AG1-IA. The isolate was deposited in the Culture Collection of Microorganisms of Environmental and Agricultural Importance, Jaguariúna, Brazil (accession number CMAA 1640).

The internal transcribed spacer (ITS) region of ribosomal DNA (primers ITS 1F/4B) was amplified and sequenced to confirm the identity of the causal agent (Gardes and Bruns 1993). The ITS sequence showed 96% homology with *R. solani* AG1 nucleotide sequences (GenBank accession Nos. FJ492099, KJ577141 and EF429212) using BLAST in the NCBI database, and deposited in GenBank (MG923316). In the phylogenetic tree, using Bayesian inference, the isolate CMAA 1640 grouped with isolates in the AG1-IA anastomosis group with a posterior probability of 0.91 (Fig. 2).

To test pathogenicity, the isolate was cultured on PDA and maintained at 25° C for 5 days. Three mycelial discs, obtained from the periphery of this colony, were transferred to 250-mL Erlenmeyer containing 100 mL of Potato Dextrose medium and incubated at $28 \pm 2^\circ$ C at 140 rpm for 8 days. After this period, the mycelial mass was filtered through cheesecloth, suspended in sterile distilled water, and ground. The concentration of the inoculum was adjusted to 1×10^5 mycelial fragments/ml with the aid of a Neubauer chamber.

To prove Koch's postulates, five plants of yard-long bean (cv. De Metro), with two or three trifoliate leaves, were sprayed with a suspension of 1×10^5 mycelial fragments/ml and 0.05% Tween® 20 until runoff. The control plants were sprayed with water and 0.05% Tween® 20. The inoculated plants were kept inside acrylic boxes, in a constant humid chamber under greenhouse conditions at 25 °C. After 4 days, all the plants inoculated showed the same symptoms initially observed in the field (Fig. 1D). Leaf samples from all symptomatic plants were plated onto PDA. The colonies were observed and had morphological characteristics identical with the isolate of *R. solani* CMAA 1640, fulfilling Koch's postulates.

For a germplasm resistance assay, an experiment was carried out in a greenhouse. Fifty nine treatments (all 58 accessions available in the germ bank from Embrapa and one control) were laid out in a completely randomized design. Six styrofoam boxes containing 128 seedlings were used for each treatment. The seedlings were inoculated after 6 days after germination using a suspension of 1×10^5 mycelial fragments/ml and 0.05% Tween® 20. The control seedlings were sprayed with water and 0.05% Tween® 20. The inoculated seedlings were kept in a constant humid chamber by using plastic bags under greenhouse conditions at 25 °C. The number of dead seedlings was assessed daily, and all 58 accessions inoculated died after nine days. The control seedlings remained asymptomatic.

Fig. 2 A Bayesian inference phylogenetic tree of ITS gene sequences showing the *Rhizoctonia solani* AG1- IA groups. The isolate of *R. solani* associated with yard-long bean CMAA 1640 (GenBank MG923316) is indicated in bold type font. Node labels indicate posterior support probability. The tree was rooted with *Ceratobasidium ramicola* (DQ278931) as an out-group

This is the first report of *Rhizoctonia solani* AG1-IA in Brazil causing mortality in yard-long bean. The disease has been reported in other two municipalities in the state of Pará with extensive negative impact for farmers. Yard-long bean is used to replace the common bean (*Phaseolus vulgaris*) crop because it is more tolerant of water deficits and the high

temperatures of the region. This makes the crop one of the most important vegetables in the Amazon region. Cultivar De Metro is the only commercially registered cultivar of yard-long bean in Brazil and it is susceptible to *R. solani*. Thus, finding accessions with resistance to this pathogen is crucial for continued successful cultivation of yard-long bean.

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