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First record of *Rhizoctonia solani* AG-7 causing root rot of common bean in Mexico

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ABSTRACT

Background/Objective. In November 2020, in two bean (*Phaseolus vulgaris*) plantations in northern Sinaloa, symptoms of root rot and plant dieback were observed, with an incidence of up to 35% per plot. The present study was established with the aim of identifying, through the sequencing of ITS and RPB2, the pathogens associated with root rot in beans**.**

Materials and Methods. Plants with symptoms were selected from bean crops in Ahome, Sinaloa, Mexico. Samples of the lesion advances of the roots of the plants were taken, and fungi with typical morphological characteristics of *Rhizoctonia* spp. were isolated, which presented septate, hyaline mycelium, forming a right angle and constriction of the basal cell, did not produce spores, and formed sclerotia. Koch's postulates were performed in pots, the inoculum was placed alongside the seed at the time of planting to evaluate the effect on germination and severity in seedlings. Once its pathogenicity was demonstrated, the isolates FAVF397 and FAVF398 were identified molecularly.

Results. The isolates FAVF397 and FAVF398 were molecularly identified as *Rhizoctonia solani* AG-7 and are responsible for the root rot of common bean.

Conclusion. The AG7 anastomosis group is reported for the first time in beans in Sinaloa. These findings represent a scientific contribution for the benefit of producers of this crop by allowing the design of relevant and effective management strategies.

Keywords: *Phaseolus vulgaris*, ITS, RNA polymerase II RPB2

INTRODUCTION

In Mexico, over a million hectares of common beans (*Phaseolus vulgaris*) are planted every year for human consumption. Production can be compromised by the incidence of diseases such as white mold, charcoal rot and rhizoctoniasis, which lead to the death of the plant.

In November 2020, symptoms of rotting were observed in two bean fields (Azufrado Higuera variety) located in Ahome, Sinaloa, Mexico. The diseased plants displayed reduced growth, dark brown canker at the base of the stem, root rot and the absence of secondary roots (Figure 1 A). The incidence of the disease in the field was estimated to be as high as 35%. For the isolation of fungi, symptomatic roots were disinfested superficially with 1% sodium hypochlorite for 2 min, rinsed twice with sterile distilled water and dried using sterile filter paper. Small pieces of diseased roots were placed in potato, dextrose and agar (PDA) medium and incubated at 25 °C for 48 h. Ten colonies, similar to *Rhizoctonia* were obtained, which were purified using the hyphal tip method. The colonies on PDA were initially white and later turned maroon (Figure 1 C and D). They hyphae were septated, from 4.6 to 5.3 µm and branched at right angles with a septum near the branching point. Microscopic examination by safranin-O staining displayed a range of 2 to 10 nuclei per cell (Figure 1 E).

The morphological characteristics of the isolates obtained coincided with those of *Rhizoctonia solani*. Two representative isolates were used for the molecular and pathogenicity tests. The isolates were placed in the Collection of Phytopathogenic Fungal Cultures of the School of Agriculture at Valle del Fuerte, Universidad Autónoma de Sinaloa (Accession Nos. FAVF397 and FAVF398). For molecular identification, the genomic DNA was extracted from each isolate, and the internal transcribed spacer (ITS) region and partial fragments of the second largest subunit of the polymerase RNA II gene (RPB2) were aplified and sequenced using the pair of primers ITS5 /ITS4 (White *et al*., 1990) and RBP2-980F/RPB2-7cR (Liu *et al*., 1999), respectively. The sequences were placed in the GenBank (accession numbers OR590793 and OR590801 for ITS and SUB14697714.1 and SUB14697714.2 for RPB2). A phylogenetic tree based on maximum-likelihood, which included combined data from the ITS and RPB2 sequences published for diverse anastomosis groups (AG) of *Rhizoctonia solani*. The phylogenetic tree clustered isolates FAVF397 and FAVF398 within the AG 7 clade (Figure 2). The pathogenicity tests for each isolate were performed with the inoculation of 10 healthy common bean seedlings (aged 15 days) planted in pots. A total of 50 ml of a mycelium una suspension adjusted at a concentration of 1×10^5 mycelial fragments/ml were placed directly on the base of the stem of each plant. Five non-inoculated common bean plants were used as a control. All bean plants were kept in a greenhouse for 15 days at temperatures ranging between 22 and 32 °C. The symptoms of root rot and stem canker were observed in the inoculated plants after 30 days (Figure 1 B). Meanwhile, the control seedlings remained asymptomatic. The pathogenicity test was performed twice with similar results. The fungi were reisolated from the infected roots and were found to be morphologically identical to the isolates used for inoculation, thus fulfinning Koch's postulates (Volci, 2008).

Consecuently, identification by morphology and sequence analysis confirmed that the causal organism of *R. solani* AG-7. This pathogen was first reported in cotton in Georgia, U.S.A. and Egypt (Baird *et al*., 1997; 2000; Abd-Elsalam *et al*., 2009), as well as in soybean

in Taiwan (Yu-Cheng *et al*., 2021) and potato in Mexico (Carling *et al*., 1998). This is the first report of *Rhizoctonia solani* AG-7 causing root rot in bean in Mexico. This report will help create new strategies for the management of the disease in bean plants.

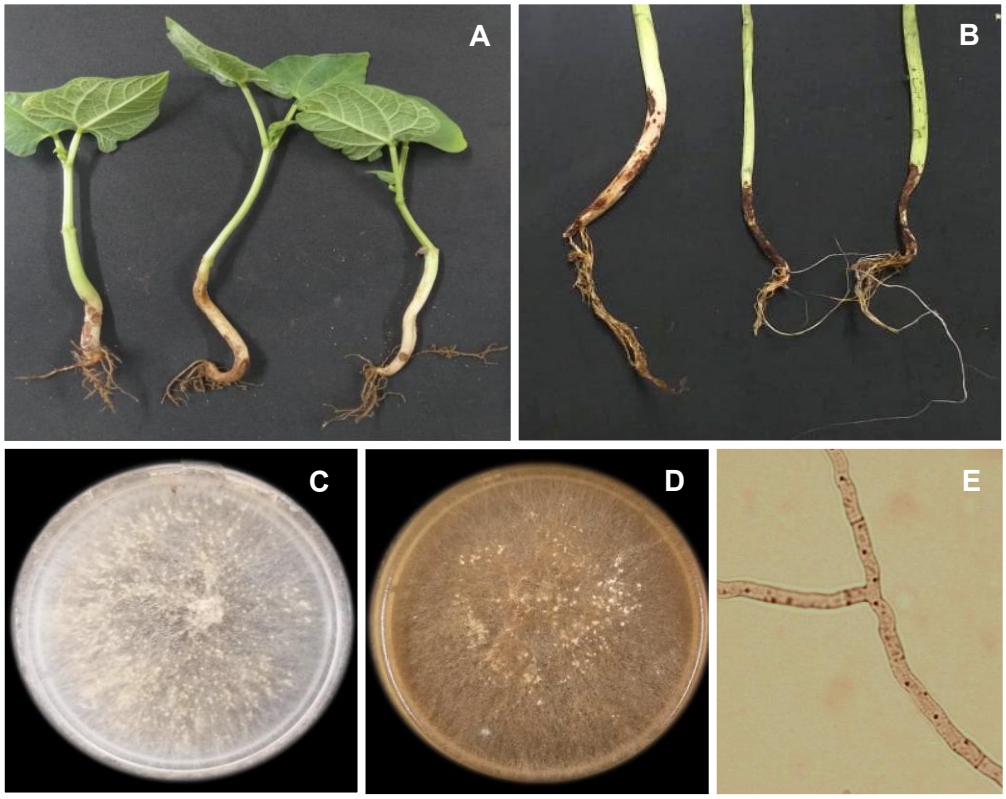

Figure 1. *Rhizoctonia solani* AG-7 causing root rot of the common bean. **A**. Symptoms on naturally infected stems and roots. **B**. Symptoms in artificially inoculated plants, 30 days after inoculation. **C**. Colony on PDA after 7 days at 25 °C in the dark. **D**. Colony on PDA after 20 days. **E**. Hyphae stained with Safranin-O showing multinucleated cells and branching at right angles, slightly constricted at their base.

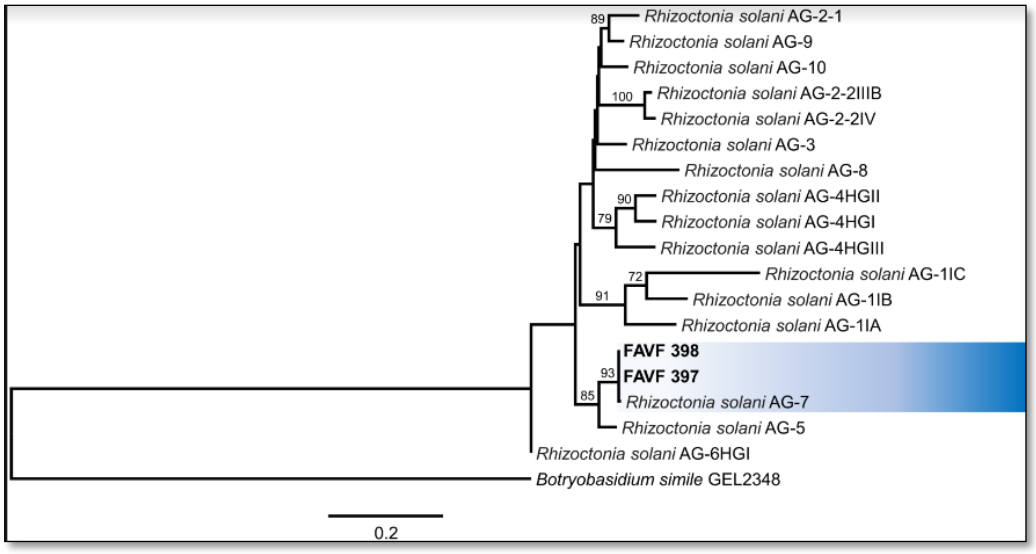

Figure 2. Phylogeny by MV of the concatenated sequences of the ITS and RPB2 genes from two isolates of *Rhizoctonia* spp. obtained from bean plants with symptoms of wilting and root rot. Bootstrap of 1000 replicas; outgroup: Botryobasidium simile. AG=Anastomosis group.

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