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Molecular identification and pathogenicity of *Rhizoctonia* spp. recovered from seed and soil samples of the main bean growing area of Argentina

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Abstract

The objective of this study was to perform the molecular and pathogenic characterization of *Rhizoctonia solani* in bean seed and soil samples from fields with root rot symptoms in northwestern Argentina. Fifty-two *Rhizoctonia* spp. isolates were collected in ten naturally infested bean fields and characterized by morphological traits, DNA sequencing, and pathogenicity. The mycelium color, the pattern of sclerotia formation, and the number of nuclei per hyphal cell were determined for each isolate. According to the variability in the rDNA-ITS region, isolates were identified as *R. solani* (85%), *Waitea circinata* var. *zeae* (*Rhizoctonia zeae*) (2%) and *Rhizoctonia* spp. (13%). Most isolates of *R. solani* (92%) were found to belong to the anastomosis group (AG) AG 4, including seven AG 4 HG-I and nine AG 4 HG-III isolates. AGs obtained from soil samples were more variable than those obtained from seed samples. Molecular identification of the isolates was in agreement with their morphological characterization. In addition, aggressiveness of the isolates towards bean seedlings was assessed in the greenhouse. Four virulence categories were defined according to the disease reaction on root and foliar tissues, which showed great variability in virulence among the isolates. Our results suggest that both seed and soil-borne inoculum may play a significant role in pathogen dispersal in the region. This is the first study on *Rhizoctonia* species and AGs in bean seed and soil in this region and it may contribute towards an efficient control strategy for bean diseases caused by *Rhizoctonia* species.

Keywords: anastomosis group, rDNA-ITS, *Phaseolus vulgaris*, root rot.
Abbreviations: AG_anastomosis group, DSI_disease severity index, HV_highly virulent, ITS_internal transcribed spacer, MV_moderately virulent, NV_non virulent, WV_weakly virulent.

Introduction

Common bean (*Phaseolus vulgaris* L.) represents an important source of protein and carbohydrates to the diet of many African and Latin American countries (Broughton et al., 2003). Argentina is among the five major producers of common bean worldwide, exporting 98% of its production. The cultivars are mainly white (Alubia) and black beans (85%), and the main cultivated areas are concentrated in the northwestern provinces of the country, with 90% of the total common bean produced in Salta province.

Root rot caused by the fungus *Rhizoctonia solani* Kühn [teleomoph *Thanatephorus cucumeris* (Frank) Donk)] is among the major diseases affecting common bean in Argentina and other bean growing areas globally (Abawi, 1989; Mathew and Gupta, 1996; Valenciano, 2006; Naseri, 2008), particularly in regions with low soil fertility, characterized by limited crop rotation and intensive seasonal bean production (Micklas et al., 2006). High inoculum levels of the pathogen affect seed germination and seedling development. *Rhizoctonia* root rot symptoms are characterized by reddish-brown, sunken lesions on seedling stems and roots and non-germination of severely infected seeds (Abawi, 1989). The rapid expansion of lesions in young seedlings often results in damping-off. Considerable yield losses, as high as 76%-100%, have been reported (Abawi, 1989; Naseri 2008). Variation in yield losses from one season to another and among fields of the same area is affected in part by environmental and soil conditions at planting time and by inoculum density (Abawi, 1989).

The *Rhizoctonia* root rot pathogen has a facultative parasitic ability and can survive as a saprotroph as mycelium and sclerotia in colonized plant residues. It can also be free in soil and disseminated by wind or water. Recurring disease cycles increase inoculum in the soil, resulting in higher fungal densities. Thus, contact with the hosts is enhanced, giving rise to an increase in primary infection. The pathogen also survives on seeds playing a significant role in long distance and overwintering dispersal (Abawi, 1989; Schwartz et al., 2005). During 2012, the percentage of infested seeds in common bean cultivars harvested from naturally infested fields in Salta province, Argentina, was as high as 36%

(Benedettini, 2012). However, the seed and soil-borne pathogen causing root rot in cultivated bean fields in Argentina has not been thoroughly identified.

The identification of the *Rhizoctonia solani* complex is based on the mycelial compatibility between isolates, which makes it possible to assign them to genetically isolated anastomosis groups (AGs) (Sneh et al., 1991; Carling 1996).Recently, 13 AGs have been reported (Liu et al., 1993; Carling 1996; Tu et al., 1996; Carling et al., 2002; Sharon et al., 2008). Some of them were further divided into subgroups based on cultural characteristics, epidemiological differences as well as rDNA-ITS and RFLP sequence analyses (Godoy-Lutz et al., 2003, 2008; Kiliçoğlu and Özkoç, 2008, 2013; Spedaletti et al., 2016). Root and hypocotyl rot are mainly caused by isolates of *R. solani* AG 1, AG 2, AG 4, and AG 5 (Galindo et al., 1982; Win and Sumner, 1987; Abawi, 1989; Tu et al., 1996; Eken and Demirci, 2004; Nerey et al., 2010; Valentin et al., 2016).

The most effective strategy for controling *Rhizoctonia* root rot is the use of genetic resistance. However, implementing compatible and effective disease control measures may be an alternative until a resistant cultivar adapted to the region becomes available (Abawi, 1989). Considering that seed and soil-borne inoculum may play a significant role in *R. solani* dispersal, the ability to detect the presence of the pathogen in the seed or the soil provides valuable information to implement management strategies that reduce the spread of the disease. This study focuses on the molecular and pathogenic characterization of the root and hypocotyl rot pathogen *Rhizoctonia solani* recovered from seed and soil samples from naturally infested common bean fields in northwestern Argentina.

Results

Morphological characterization

A total of 54 isolates were collected from different bean fields with white and black bean cultivars in Salta and Tucumán provinces, northwestern Argentina. Twenty-two of these isolates were recovered from bean seeds and 32 from soil samples (Table 1). According to cultural characteristics, 38 isolates were identified as *Rhizoctonia solani* and the rest as *Rhizoctonia* spp. The latter included one isolate (Rs67) identified as *Rhizoctonia zeae* (*Waitea circinata* var *zeae*). The mycelium color varied from white to light brown or light orange. In young *R. solani* colonies, mycelium was initially white and turned brown or light brown in mature cultures. Different patterns of sclerotia formation were observed: central, peripheral, and scattered sclerotia, and no sclerotia formation. Poor mycelial growth was observed after two weeks of incubation at 25±2 °C. Besides, *R. zeae* colonies were light orange and produced abundant aerial mycelium. All the isolates presented multinucleate hyphal cells with an average of five nuclei per cell.

Molecular characterization

Amplification of the ITS regions with ITS1/ITS4 primers yielded a DNA fragment of about 700 bp and uniform size among all isolates tested (Supplementary Figure 2). PCR products were sequenced and the ITS sequences were deposited in GenBank (Table 1). Sequence polymorphism was observed within the ITS1 and ITS2 regions (Supplementary Figure 3). The phylogenetic tree generated on the basis of the ITS sequences showed that the isolates tested and the sequences retrieved from GenBank were grouped distinctly according to their AG (Figure 1). The isolates molecular identification was in agreement with their morphological characterization. Thirty-five of the *R. solani* isolates (92%) were characterized as AG 4, including seven AG 4 HG-I and nine AG 4 HG-III isolates. One AG 2-1 and two AG 7 isolates were also identified.

The isolates obtained from soil samples were more variable than those obtained from seeds. The 25 isolates recovered from soil samples resulted in 17 isolates identified as AG 4 and one as AG 2-1 (Table 1). In addition, one isolate corresponded to *Rhizoctonia zeae* (*Waitea circinata* var. *zeae*) and six isolates were included in the cluster with *Rhizoctonia* sp. (KC176298) (Figure 1). On the other hand, the twenty isolates obtained from seeds and analyzed by means of rDNA-ITS sequencing were identified as *R. solani*, including 18 isolates of AG 4 and two of AG 7.

Pathogenicity assay

Most of the isolates included in the pathogenicity test were able to cause typical web blight and root rot symptoms (Table 2). Ninety-six percent of the isolates tested were virulent to common bean and produced typical root rot symptoms (Table 2). Only isolate Rs14 was non-pathogenic under the conditions of the test (DSI = 1). Significant differences in aggressiveness were observed among isolates ($F= 18.28$, $P < 0.0001$). The isolates were grouped into four virulence categories based on disease reaction on root tissues: non virulent (1), weakly virulent (2), moderately virulent (14), and highly virulent (5) (Table 2). The five highly virulent isolates (Rs88, Rs82, Rs86, Rs77, and Rs89) showed DSI values ≥ 7.00, with more than 50% of the hypocotyl and root tissues with symptoms. All of these isolates were obtained from soil samples collected in bean fields from San Agustín, Tucumán province, except for Rs77, which was collected from Tartagal, Salta province. All the fields sampled presented moderately virulent isolates, while the two weakly virulent isolates were from Tartagal, Salta province. All isolates tested caused typical web blight symptoms after four days of inoculation (Table 2) but all of them showed low disease severity ratings ($1 < DSI < 7$).

Discussion

In this study, 54 *Rhizoctonia* spp. isolates obtained from seed and soil samples collected in common bean fields in northwestern Argentina were characterized as *Rhizoctonia solani* (70 %) and *Rhizoctonia* spp. (30 %) by morphological traits, DNA sequencing, and pathogenicity approaches.

Root rot caused by *Rhizoctonia solani* is a widely distributed disease in common bean in the region and has caused severe damage in the last crop seasons (Vizgarra et al., 2014; Mamani-Gonzales, 2015). However, *Rhizoctonia* isolates associated with bean root rot as a source of inoculum in common bean fields in Argentina had never been characterized before. Determining the association of seed and soil-borne pathogens with disease epidemics requires comprehensive information on the pathogens involved in order to develop efficient control methods. This study revealed the presence of various *R. solani* AGs in seed and soil samples from bean fields naturally infested with root rot in northwestern Argentina. Our results showed that 92 % of the *R. solani* isolates analyzed were identified as AG 4. Furthermore, the molecular characterization of the ITS region made it possible to identify some AG 4 isolates to the anastomosis subgroup level, resulting in AG 4 HG-I (20 %) and AG 4 HG-III (26 %). All of these isolates were found to

Table 1. Anastomosis group (AG), isolate code, origin, source and GenBank accession number of 54 isolates of *Rhizoctonia* spp. recovered from seed and soil samples collected in ten fields in northwestern Argentina during the 2012–2014 growing seasons.

AG	Isolate	Location/Province	Source	Accession number
Rhizoctonia solani				
AG 2-1	Rs86	San Agustín/Tucumán	Soil	KP736196
AG 4	Rs10	Gral. San Martín/Salta	Seed	KF686790
AG 4	Rs11	Gral. San Martín/Salta	Seed	KF686791
AG 4	Rs26	Gral. San Martín/Salta	Seed	KF686797
AG 4	Rs28	Pichanal/Salta	Seed	KF686798
AG 4	Rs47	Gral. San Martín/Salta	Seed	KF686806
AG 4	Rs5	Gral. San Martín/Salta	Seed	KF686785
AG 4	Rs19	Gral. San Martín/Salta	Seed	KF686795
AG 4	Rs20	Gral. San Martín/Salta	Seed	KF686796
AG 4	Rs6	Gral. San Martín/Salta	Seed	KF686786
AG 4	Rs14	Pichanal/Salta	Seed	KF686793
AG 4	Rs18	Gral. San Martín/Salta	Seed	KF686794
AG 4	Rs84	Tartagal/Salta	Soil	KP736195
AG 4	Rs79	Tartagal/Salta	Soil	KP736193
AG 4	Rs74	Tartagal/Salta	Soil	KP736188
AG 4	Rs75	Tartagal/Salta	Soil	KP736189
AG 4	Rs76	Tartagal/Salta	Soil	KP736190
AG 4	Rs77	Tartagal/Salta	Soil	KP736191
AG 4	Rs81	San Agustín/Tucumán	Soil	KP736194
AG 4	Rs56	Tartagal/Salta	Soil	KP736174
AG 4 HG-I	Rs1	Tucumán	Seed	KF686782
AG 4 HG-I	Rs2	Tucumán	Seed	KF686783
AG 4 HG-I	Rs30	Gral. San Martín/Salta	Seed	KF686800
AG 4 HG-I	Rs31	Gral. San Martín/Salta	Seed	KF686801
AG 4 HG-I	Rs12	Gral. San Martín/Salta	Seed	KF686792
AG 4 HG-I	Rs58	Tartagal/Salta	Soil	KP736176
AG 4 HG-I	Rs87	San Agustín/Tucumán	Soil	KP736197
AG 4 HG-III	Rs43	Gral. San Martín/Salta	Seed	KF686804
AG 4 HG-III	Rs44	Gral. San Martín/Salta	Seed	KF686805
AG 4 HG-III	Rs52	Tartagal/Salta	Soil	KP736173
AG 4 HG-III	Rs57	Tartagal/Salta	Soil	KP736175
AG 4 HG-III	Rs59	Tartagal/Salta	Soil	KP736177
AG 4 HG-III	Rs62	Tartagal/Salta	Soil	KP736178
AG 4 HG-III	Rs64	Tartagal/Salta	Soil	KP736180
AG 4 HG-III	Rs63	Tartagal/Salta	Soil	KP736179
AG 4 HG-III	Rs65	Tartagal/Salta	Soil	KP736181
AG 7	Rs40	Gral. San Martín/Salta	Seed	KF686802
AG 7	Rs42	Gral. San Martín/Salta	Seed	KF686803
Waitea circinata var. *zeae*				
--	Rs67	Tartagal/Salta	Soil	KP736182
Rhizoctonia sp.				
--	Rs68	Tartagal/Salta	Soil	KP736183
--	Rs69	Tartagal/Salta	Soil	KP736184
--	Rs51	Tartagal/Salta	Soil	KP736172
--	Rs70	Tartagal/Salta	Soil	KP736185
--	Rs71	Tartagal/Salta	Soil	KP736186
--	Rs72	Tartagal/Salta	Soil	KP736187
--	Rs15	Pichanal/Salta	Seed	--
--	Rs16	Pichanal/Salta	Seed	--
--	Rs78	Tartagal/Salta	Soil	--
--	Rs88	San Agustín/Tucumán	Soil	--
--	Rs89	San Agustín/Tucumán	Soil	--
--	Rs90	San Agustín/Tucumán	Soil	--
--	Rs82	San Agustín/Tucumán	Soil	--
--	Rs73	Tartagal/Salta	Soil	--
--	Rs85	Tartagal/Salta	Soil	--

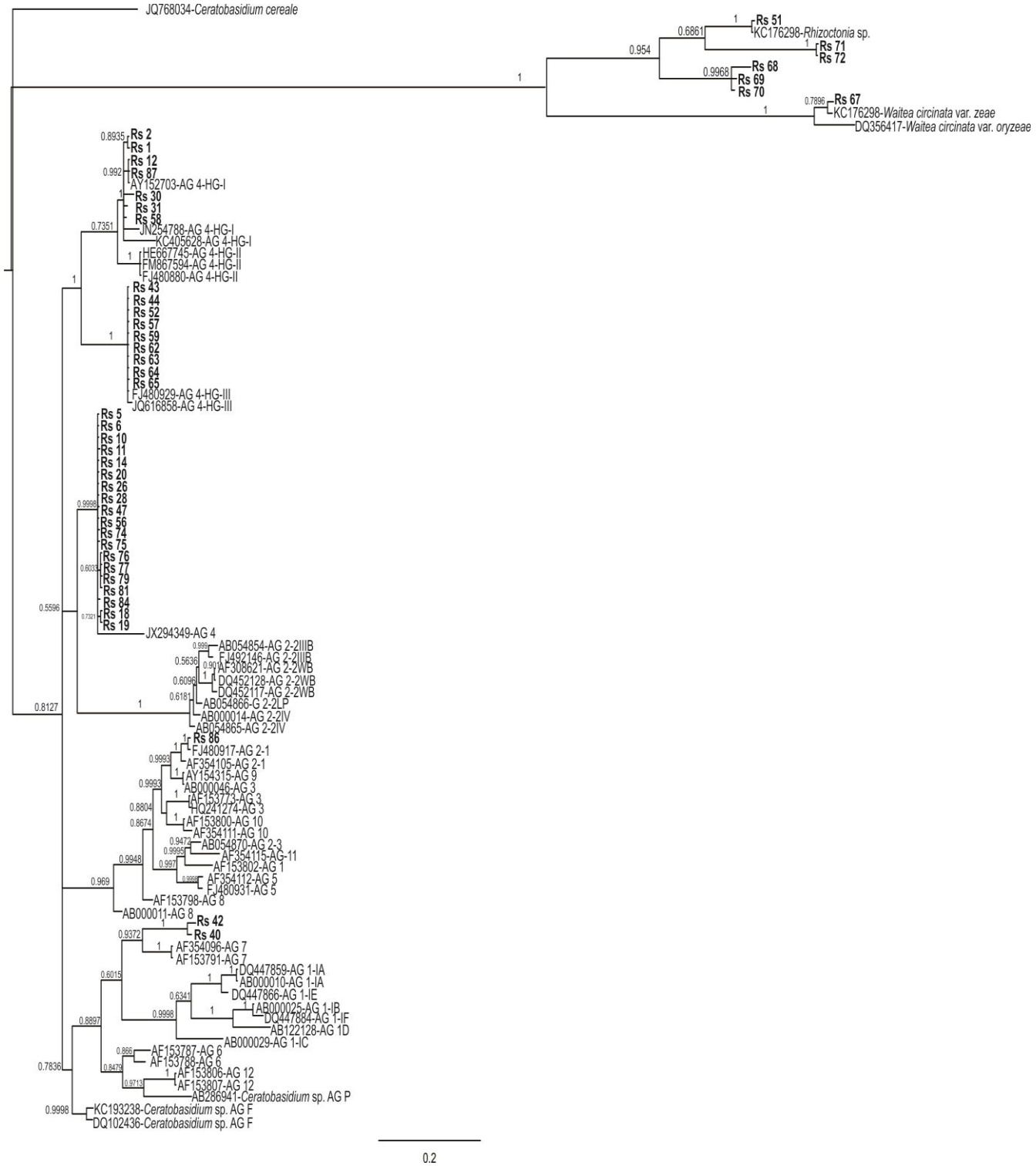

Fig 1. Phylogenetic tree based on rDNA-ITS sequences of *Rhizoctonia* spp. isolates recovered from seed and soil samples collected in ten fields with plants showing symptoms of root and hypocotyl root from northwestern Argentina, and corresponding sequences from GenBank. Numbers above the branches indicate node support (posterior probability). The scale bar represents the number of substitutions per site. Sequences obtained in this study are shown in bold. *Ceratobasidium cereale* was used as outgroup.

Table 2. Root rot (RR) and web blight (WB) disease severity index (DSI) of 22 *Rhizoctonia* spp. isolates collected in different fields in northwestern Argentina.

Isolate	AG[a]	RR DSI[b]	RR Virulence[c]	WB DSI[d]	WB Virulence[c]
Rs86	AG 2-1	7.20	HV	2.89	WV
Rs77	AG 4	7.00	HV	1.92	WV
Rs76	AG 4	6.82	MV	1.67	WV
Rs79	AG 4	6.54	MV	1.83	WV
Rs18	AG 4	4.43	MV	1.33	WV
Rs81	AG 4	3.75	MV	3.41	MV
Rs74	AG 4	3.00	WV	3.67	MV
Rs75	AG 4	3.17	MV	3.42	MV
Rs14	AG 4	1.00	NV	1.08	WV
Rs87	AG 4 HG-I	5.22	MV	3.11	MV
Rs12	AG 4 HG-I	3.67	MV	1.17	WV
Rs64	AG 4 HG-III	4.20	MV	4.11	MV
Rs62	AG 4 HG-III	3.00	WV	5.22	MV
Rs42	AG 7	6.23	MV	1.83	WV
Rs88	Nd	7.86	HV	4.33	MV
Rs82	Nd	7.57	HV	4.78	MV
Rs89	Nd	7.00	HV	4.78	MV
Rs78	Nd	6.80	MV	1.08	WV
Rs16	Nd	6.45	MV	4.17	MV
Rs85	Nd	6.14	MV	3.89	WV
Rs71	Nd	5.83	MV	1.25	WV
Rs15	Nd	4.57	MV	Nd	Nd

[a] Anastomosis group; Nd: not determined.
[b] Disease severity index mean of root rot score based on a 1-9 scale; LSD (0.05): 2.56. Overall mean: 5.33.
[c] NV: non virulent (DSI = 1); WV: weakly virulent (1 < DSI ≤ 3); MV: moderately virulent (3 < DSI< 7); HV: highly virulent (DSI ≥ 7).
[d] Disease severity index mean of web blight score based on a 1-9 scale; LSD (0.05): 1.91. Overall mean: 2.89.

be virulent to common bean in the pathogenicity test performed under controlled conditions. AG 4 had previously been reported to be the prevalent group associated with root and hypocotyl rot in other common bean growing areas worldwide (Muyolo et al., 1993; Meinhardt et al., 2002; Nerey et al., 2010; Kiliçoğlu and Özkoç, 2013; Haratian et al., 2013). In Zaire, isolates obtained from diseased dry bean roots and hypocotyls were characterized as AG 4 based on anastomosis-group determination using tester strains (Muyolo et al., 1993). Most of the *R. solani* isolates recovered from bean plants showing root and hypocotyl rot symptoms collected in Brazil, Cuba, Iran and Turkey were characterized as AG 4 HG-I based on different techniques including sequencing of the rDNA-ITS region (Meinhardt et al., 2002; Nerey et al., 2010; Kiliçoğlu and Özkoç, 2013; Haratian et al., 2013). AG 4 isolates have also been reported to be associated with web blight in common bean (Gálvez et al., 1989; Tu et al., 1996; Godoy-Lutz et al., 2003, 2008; Yang et al., 2007; Dubey et al., 2014). Moreover, *R. solani* AG 4 can attack other commercial crops including maize and tobacco (Bacharis et al., 2010; Mercado-Cárdenas et al., 2015), which are grown in rotation with bean in northwestern Argentina. Isolates identified as *R. solani* AG 4 HG-I and AG 4 HG-III were obtained from tobacco plants with sore shin and damping-off symptoms in different fields in the Lerma Valley, Salta province (Mercado-Cárdenas et al., 2015). The use of non-host crops in rotational systems may lead to improved control, thus reducing the incidence of bean root rot. Nevertheless, these systems do not completely eradicate the pathogen (Abawi, 1989). All the isolates included in the pathogenicity assay, except for Rs14, were able to produce typical root rot and web blight symptoms. Symptoms on leaf tended to be less severe than those on root in most of the isolates evaluated (85%). The ability of specific isolates within a single AG to overcome tissue-specific resistance mechanisms causing more than one disease in common bean has recently been reported (Valentín-Torres et al., 2016). AG 4 isolates collected from bean leaves and roots were able to induce root rot and web blight symptoms in common bean plants (Valentín-Torres et al., 2016), and root rot readings were more severe than web blight readings. However, a more thorough study including isolates obtained from bean plant tissues showing root rot symptoms would be necessary to obtain comparable results. In addition, a more structured sampling of the isolates in different regions would make it possible to identify the effect of edaphic and climatic conditions on the population dynamics of the pathogen and to elucidate the relationship of *R. solani* inoculum density with the incidence of bean root and hypocotyl rot in the field of the studied region. One *Rhizoctonia solani* isolate from soil and one from bean seeds identified as AG 2-1 and AG 7 caused root rot and web blight symptoms in our inoculation trials. There are reports of AG 2-1 and AG 7 isolates causing disease in tobacco and cotton (Bacharis et al., 2010; Mercado-Cárdenas et al., 2012), but not on bean plants, as we observed under controlled environment conditions. Moreover, AG 2-1 isolates were found to be associated with damping-off, target spot, and sore shin in tobacco in the same area (Mercado-Cárdenas et al., 2015). Fifty-three and 47 % of the *Rhizoctonia solani* isolates were obtained from seed and soil samples, respectively, suggesting that both, seed and soil-borne inoculum may play a significant role in pathogen dispersal in the region, as reported in other areas worldwide (Naseri and Mousavi, 2015). The use of certified seed free of sclerotia is one of the keys to reducing the incidence of root rot disease. In this way, the information generated in the present study could be useful in developing new sensitive methods for pathogen detection based on the polymorphism detected in the DNA sequences.

Materials and Methods

Fungal isolates

During 2012-2014, *Rhizoctonia* spp. isolates were recovered from seed and soil samples collected in ten fields with plants

showing symptoms of root and hypocotyl rot from four locations in Salta and Tucumán provinces, northwestern Argentina (Supplementary Figure 1). Isolation from seeds was made on potato dextrose agar (PDA). Four hundred seeds from each field were surface sterilized (70% EtOH for 2 min; 5% NaClO for 2 min), rinsed twice in sterilized distilled water, and plated on 2% PDA acidified to pH 5 with 10% lactic acid. Ten seeds were sown per plate and incubated at 24 ± 2°C in darkness for 3 days. Hyphal tips were transferred to a new medium and the cultures were examined microscopically for morphological characters (Sneh et al., 1991).

Five soil samples, weighing 1 kg each, were collected at 0–10 cm depth from around the roots of five plants per field. Samples were air-dried and ground to pass through a 1-mm sieve before use. Isolation from soil was done as described elsewhere (Alfenas et al., 2007). Briefly, 100 g of soil was transferred to sterile 15-cm diameter dishes and moistened with sterile water. The soil moisture content was maintained at <30±45 % (w/w). Segments of eucalyptus branches were sterilized twice in the autoclave (120 °C for 30 min), on two successive days and added to the soil. After incubation at 25 °C for 24–48 h, segments were surface sterilized (70 % EtOH for 2 min; 1 % NaClO for 2 min), transferred to new dishes with PDA, and incubated at 25±2°C in darkness for 12–24 h. Fungal colonies morphologically similar to *Rhizoctonia* spp. were transferred onto PDA. Pure cultures were obtained by transferring hyphal tips to new dishes of PDA and identified according to morphological features and DNA characterization (Sneh et al., 1991).

Morphological characterization

Isolates were morphologically characterized from cultures grown on PDA at 25 ± 2°C in darkness for 20 days using taxonomic keys (Watanabe, 2002; Barnett and Hunter, 2006). The mycelium color and the pattern of sclerotia formation in the culture plates were registered for each isolate. The number of nuclei per hyphal cell was determined by means of the nuclear-staining procedure using acridine orange, as described by Sneh et al. (1991) and observed by fluorescence microscopy.

DNA extraction, amplification and sequencing

Genomic DNA was extracted from 250 mg of hyphal tissue using a SDS protocol. The rDNA-ITS region was amplified using primers ITS1 and ITS4 (White et al., 1990). The PCR reactions were carried out in a 50-µl final volume containing 12–15 ng of genomic DNA, 1x reaction buffer (100 mM Tris–HCl [pH 9.0], 500 mM KCl, 1% Triton® X-100), 0.1 µM of each primer (Genbiotech S.R.L. Buenos Aires, Argentina), 200 µM of each dNTP, 3.0 mM $MgCl_2$ and 1.0 unit of *Taq* DNA polymerase (Highway-Inbio, Tandil, Argentina). The DNA amplifications were performed using a Eppendorf Master Cycler Gradient thermocycler (Hamburg, Germany) programmed with an initial denaturing step at 94°C for 1 min, followed by 30 cycles at 94°C for 15 s, 58°C for 15 s, and 72°C for 15 s, and a final extension cycle at 72°C for 7 min. A 10-µl aliquot of the PCR product was resolved by electrophoresis through 1.5% (w/v) agarose gels stained with GelRed™ (Biotium, Hayward, CA, USA) at 90 V in 1x TBE buffer for 1 h at room temperature. The fragments were visualized under UV light. The size of the DNA fragments was estimated by comparison with a 100-1000 bp DNA ladder (Highway-Inbio, Tandil, Argentina).

The fragments were purified and subjected to sequencing in both directions using primers ITS1 and ITS4 on the 3500xL Genetic Analyzer sequencer (Applied Biosystems) at the Biotechnology Institute of INTA (Castelar, Buenos Aires, Argentina). Sequences were aligned using the CLUSTALW algorithm (Thompson et al., 1994) and adjusted by eye. Ambiguous portions of the alignment were deleted. Phylogenetic tree was inferred using Bayesian inference as implemented in MrBayes v. 3.1.6 (Ronquist et al., 2012). The general time reversible (GTR) model with among-site substitution-rate heterogeneity described by a gamma distribution and a fraction of sites constrained to be invariable (GTR + I + G) was selected as the model of DNA substitution that best fitted the data with JModelTest 2.1.7 (Posada, 2008; Darriba et al., 2012). Two independent analyses were run with a random starting tree over 2,000,000 generations, with a sample frequency of 500, and a burn-in of 1000 trees. The tree space was explored using four chains: one cold and three incrementally heated chains. We applied several tests to assess convergence of the cold Markov chain for all MRBAYES analyses implemented in TRACER v. 1.6 (Rambaut et al., 2014), in addition to the standard deviation of the split frequencies. All posterior samples of a run prior to the burn-in point were discarded. Remaining trees were taken into account to obtain a 50% majority-rule consensus tree and mean branch length estimates. The frequency of all bipartitions was estimated to assess the support of each node (Huelsenbeck and Ronquist, 2001).

Pathogenicity determination

For the pathogenicity analysis, between three and five isolates were randomly selected from each sample site. Pathogenicity tests for 22 isolates were performed separately using black bean seedlings (cv. NAG12) grown for V3 at 25 ± 2°C with a 12-h photoperiod. Colonized wheat grains were used as the source of inoculum. The grains were moistened with distilled water and sterilized thrice in the autoclave (120°C for 30 min). The grains were transferred to a 5-day-old culture grown on PDA and incubated in darkness at 25 ± 2°C for two weeks. Twelve plants were inoculated by depositing wheat grains colonized with *Rhizoctonia solani* onto leaves or soil in contact with the stem. Plants inoculated with sterile wheat grains served as controls. The plants were placed in a 25 ± 2°C growth chamber, misted and covered with polyethylene bags. These bags were removed after 24 hours, when the plants were moved to a glasshouse. Four days after inoculation, disease severity index (DSI) was rated for leaves using the scale proposed by van Schoonhoven and Pastor-Corrales (1987): 1= No visible disease symptoms; 3= 5-10% of the leaf area with symptoms; 5= 20-30% of the leaf area with symptoms; 7= 40-60% of the leaf area with symptoms; and 9= > 80% of the leaf area with symptoms. Twelve days after inoculation, DSI was rated for hypocotyl and roots using the scale proposed by van Schoonhoven and Pastor-Corrales (1987): 1= No visible disease symptoms; 3= Light discoloration either without necrotic lesions or with 10 % of the hypocotyl and root tissues covered with lesions; 5= 25% of the hypocotyl and root tissues covered with lesions but tissues remain firm with deterioration of the root system; 7= 50 % of the hypocotyl and root tissues covered with lesions combined with considerable softening, rotting, and reduction of the root system; 9= 75 % or more of the hypocotyl and root tissues affected with advanced stages of rotting combined with a severe reduction in the root system. Analysis of variance was performed, and means were compared using Fisher's protected least significant difference

test (LSD) (P = 0.05) in the Infostat statistical software (Di Rienzo et al., 2014). Re-isolations were made from the plants showing symptoms to confirm the pathogenic nature of the isolates. All non-inoculated plants remained healthy. Depending on the DSI rates, the isolates were classified into four virulence categories: non virulent (DSI = 1), weakly virulent (1 < DSI ≤ 3), moderately virulent (3 < DSI < 7), and highly virulent (DSI ≥ 7).

Conclusion

This is the first report on the *Rhizoctonia* species and AGs present in bean seed and soil samples collected in fields with bean root rot symptoms in northwestern Argentina. Our results suggest that both seed and soil-borne inoculum may play a significant role in pathogen dispersal in the region. Confirming that these sources of inoculum are potential causes of disease is important for integrated crop management, in particular for taking preventive measures at planting time. The identification and pathogenicity determination of *Rhizoctonia* isolates, as described in this study, are the first steps towards an efficient control strategy for bean diseases caused by *Rhizoctonia* species.

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