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Genetic Characterization and Pathogenicity of *Rhizoctonia solani* Associated with Common Bean Web Blight in the Main Bean Growing area of Argentina

Yamila Spedaletti[1,2], Mónica Aparicio[1,2], Guadalupe Mercado Cárdenas[2], Marcela Rodriguero[3], Gisel Taboada[1,2], Carla Aban[1,2], Silvia Sühring[4], Oscar Vizgarra[5] and Marta Galván[1,2]

1 Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), CCT-Salta. Av. Bolivia 5150 (4400), Salta, Argentina
2 Instituto Nacional de Tecnología Agropecuaria (INTA) EEA Salta, Ruta Nac. 68 Km 172 (4403), Cerrillos, Salta, Argentina
3 Departamento de Ecología, Genética y Evolución, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, , IEGEBA (CONICET-UBA), Ciudad Autónoma de Buenos Aires, Argentina
4 Facultad de Ciencias Naturales, Universidad Nacional de Salta, Av. Bolivia 5150, Salta, Argentina
5 Estación Experimental Agroindustrial Obispo Colombres (EEAOC), Tucumán, Argentina

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Correspondence
M. Galván, Instituto Nacional de Tecnología Agropecuaria (INTA) EEA Salta, Ruta Nac. 68 Km 172 (4403), Cerrillos, Salta, Argentina.
E-mail: martazgalvan@gmail.com

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Abstract

Common bean web blight (WB), caused by the fungus *Rhizoctonia solani* (teleomorph *Thanatephorus cucumeris*), is among the endemic fungal diseases of major impact in north-western Argentina (NWA). This study aimed to analyse the genetic and pathogenic diversity of *R. solani* in Salta, NWA, where 97 isolates were recovered from commercial bean cultivars and wild beans showing WB symptoms in a major bean production area. The isolates were characterized on the basis of specific primers, rDNA-ITS sequences and morphological characteristics. All the isolates were identified as *R. solani* AG 2-2WB, and they exhibited considerable intragroup variation. The phylogenetic tree generated with the ITS sequences confirmed the isolates identification. Aggressiveness of the isolates towards bean seedlings was assessed in the greenhouse. A great variability in virulence was observed among the isolates analysed. On the basis of the disease reaction on foliar tissues, the isolates were grouped into three virulence categories as follows: weakly virulent (30%), moderately virulent (38%) and highly virulent (32%). However, no correlation between virulence and geographical origin was detected. The information generated in this study provides initial data on the population variability of the WB pathogen in north-western Argentina and represents a valuable contribution to regional breeding programmes aimed to obtain cultivars with durable resistance.

Introduction

Common bean (*Phaseolus vulgaris* L.) is the main grain legume for human consumption worldwide, representing an important source of protein and carbohydrates in the diet of many African and Latin American countries (Broughton et al. 2003). Argentina is among the five major exporters of common bean worldwide, exporting 98% of its production. The main cultivated areas are concentrated in the northern provinces of Salta, Jujuy, Tucumán, Catamarca and Santiago del Estero. However, Salta produces 90% of the total common bean production of the country. The main cultivars are white (Alubia) and black beans (85%); other types include Cranberry and Light Red Kidney.

Some endemic fungal diseases exert a major impact on the region, the bean web blight (WB) being one of the most economically important epidemics, given its level of dispersion in the

production area. The importance of the disease has increased in recent years, becoming one of the main causes of yield losses and seed quality decrease (Ploper 1981; Vizgarra et al. 2012, 2014; Mamani et al. 2013). WB epidemics are favoured by rainy weather, high-to-moderate temperature (30–20°C) and high relative humidity (>80%) (Galvez et al. 1989). Web blight is also a limiting factor in other regions of Africa, Latin America and the Caribbean (Galvez et al. 1989; Godoy-Lutz et al. 2008; Mora-Umaña et al. 2013), where yield losses from 20 to 100% have been reported (Galvez et al. 1989; Costa-Coelho et al. 2014). The disease is spread by rain-splashed sclerotia, mycelial bridges between plants, infested soil debris, airborne basidiospores and infected seeds (Echandi 1965; Cardenas-Alonso 1989; Galvez et al. 1989). As commercial varieties with WB resistance are not available, the use of costly fungicides and minimum tillage is some of the cultural practices commonly used for the disease management (Godoy-Lutz et al. 2008; Gonzalez et al. 2012).

Web blight is caused by the fungus *R. solani* Kühn [teleomorph *Thanatephorus cucumeris* (Frank) Donk]. *Rhizoctonia solani* complex identification is based on the mycelial compatibility between isolates, which makes it possible to assign them to anastomosis groups (AGs) (Sneh et al. 1991; Carling 1996). To date, 13 AGs, with numerous subgroups, have been reported (Liu and Sinclair 1993; Carling 1996; Carling et al. 2002; Sharon et al. 2008). Isolates of AG 1, AG 2 and AG 4 have been associated with WB in common bean (Galvez et al. 1989; Tu et al. 1996; Godoy-Lutz et al. 2003, 2008; Yang et al. 2007; Dubey et al. 2014). Some of them were further divided into subgroups based on cultural characteristics, epidemiological differences and rDNA-ITS sequence analyses (AG 1-IA, AG 1-IB, AG 1-IE, AG 1-IF, AG 2-2IV, AG 2-2WB) (Godoy-Lutz et al. 2003, 2008).

Web blight isolates from different regions of Latin America and the Caribbean, where WB is endemic, were identified by means of rDNA-ITS sequence analysis (Godoy-Lutz et al. 2003, 2008). However, no studies about the identification of *R. solani* causing WB in cultivated bean fields in Argentina have been performed. Only the occurrence of AG 2-2WB causing WB in wild beans from Argentina has been reported (Godoy-Lutz et al. 2003, 2008).

Assessing the diversity of the WB pathogen in a particular geographical region is important for a successful integrated management of the disease to minimize yield losses in a context of sustainable cropping. Thus, the aim of this work was to analyse the genetic and pathogenic diversity of the *R. solani* pathogen associated with common bean WB in Salta, Argentina.

Materials and Methods

Fungal isolates

A survey was conducted in the Lerma Valley region in Salta province, north-western Argentina, so as to collect bean plants showing symptoms of WB. Five commercial bean fields were sampled following a W-pattern, and approximately 50 plants were chosen per field. Isolation of the causal agent was made from symptomatic leaves on potato dextrose agar (PDA) acidified to pH 5 with 10% lactic acid and incubated at 25 ± 2°C in darkness for 2–3 days. Hyphal tips were transferred to a new medium, and the cultures were examined microscopically for morphological characters (Sneh et al. 1991). Some isolates collected from wild bean populations (*Phaseolus vulgaris* var. *aborigineus*) growing in the Lerma Valley, Salta, Argentina, were also included in the analysis.

Morphological characterization

Isolates morphological characterization was performed by observing cultures grown on PDA at 25 ± 2°C in darkness for 20 days. The mycelium colour and the pattern of sclerotia formation in the culture plates (peripheral, central, scattered or central–peripheral) were registered for each isolate (Table 1). 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 and PCR amplification

Genomic DNA was extracted from 250 mg of hyphal tissue using a CTAB protocol.

The specific primer pair 2-2WB-F (5′ GAGCATGTG CACRCCTTG 3′) and 2-2WB-R (5′ GGAACCAA GCAYAACACC 3′) designed for *R. solani* AG 2-2WB (Godoy-Lutz et al. 2008) were used to amplify the DNA and confirm the anastomosis group of each isolate. The amplification conditions were performed as described by Godoy-Lutz et al. (2008). A 10-μl aliquot of the polymerase chain reaction (PCR) product was resolved by electrophoresis through 1.5% (w/v) agarose gels stained with GelRed™ (Biotium, Hayward,

Table 1 Isolate code, location, mycelium colour, sclerotia production, disease severity index (DSI) and virulence of 97 isolates of *Rhizoctonia solani* recovered from commercial bean cultivars and wild beans showing WB symptoms in the Lerma Valley region in Salta province, north-western Argentina

Isolate	Location[a]	Mycelium colour	Sclerotia production	DSI[b]	Virulence[c]
97[e]	Chicoana	light brown	central	4.17	MV
98[e]	Chicoana	light brown	central–peripheral	5.17	MV
99[e]	Chicoana	light brown	central–peripheral	6.17	HV
100[e]	Chicoana	light brown	central	5.17	MV
101	Chicoana	light brown	central	4.33	MV
102	Chicoana	light brown	central	6.00	HV
103	Chicoana	light brown	central	4.67	MV
104	Chicoana	light brown	central	3.33	WV
105	Chicoana	light brown	central–peripheral	5.50	HV
106[de]	Chicoana	light brown	central	3.83	MV
107[e]	Chicoana	light brown	central	3.00	WV
108[de]	Chicoana	light brown	central	3.67	MV
109[de]	Chicoana	light brown	peripheral	4.67	MV
110[e]	Chicoana	light brown	central	5.25	MV
111	Chicoana	white	scattered	5.00	MV
112[e]	Chicoana	white	scattered	ND	ND
113[e]	Chicoana	light brown	peripheral	3.33	WV
114[e]	Chicoana	light brown	peripheral	7.00	HV
115[e]	Chicoana	light brown	central	3.50	MV
116	Chicoana	light brown	central–peripheral	ND	ND
117[e]	Chicoana	light brown	peripheral	6.50	HV
118[e]	Chicoana	white	scattered	5.67	HV
119[e]	Chicoana	light brown	central	2.67	WV
120[e]	Chicoana	light brown	peripheral	6.17	HV
121[e]	Chicoana	light brown	peripheral	4.67	MV
122	R. de Lerma	light brown	central	5.67	HV
123[d]	R. de Lerma	light brown	central–peripheral	5.67	HV
124	R. de Lerma	light brown	central	3.00	WV
125	R. de Lerma	light brown	central–peripheral	5.33	MV
126	R. de Lerma	light brown	central	6.67	HV
127	R. de Lerma	light brown	central	5.33	MV
128	R. de Lerma	white	scattered	1.00	WV
129	R. de Lerma	light brown	central	6.83	HV
130[e]	R. de Lerma	light brown	central–peripheral	ND	ND
131[e]	R. de Lerma	white	central	5.17	MV
132	R. de Lerma	white	scattered	1.00	WV
133[e]	R. de Lerma	light brown	peripheral	2.67	WV
134[e]	R. de Lerma	white	central–peripheral	4.00	MV
135[d]	R. de Lerma	white	central	5.67	HV
136[e]	R. de Lerma	light brown	central–peripheral	6.00	HV
137[e]	R. de Lerma	dark brown	central–peripheral	6.67	HV
138	R. de Lerma	dark brown	central	3.00	WV
139[e]	R. de Lerma	white	central–peripheral	4.83	MV
140	R. de Lerma	dark brown	central–peripheral	5.33	MV
141[e]	R. de Lerma	light brown	central	5.76	HV
142	Cerrillos	light brown	central	7.67	HV
143	Cerrillos	light brown	peripheral	7.17	HV
144[e]	Cerrillos	dark brown	central	6.67	HV
145	Cerrillos	dark brown	central–peripheral	5.33	MV
146[e]	Cerrillos	white	scattered	3.67	MV
147[e]	Cerrillos	light brown	central	5.67	HV
148[e]	Cerrillos	dark brown	scattered	1.00	WV

(continued)

Table 1 (continued)

Isolate	Location[a]	Mycelium colour	Sclerotia production	DSI[b]	Virulence[c]
149	Cerrillos	light brown	central	2.67	WV
150	Cerrillos	light brown	central	ND	ND
151[e]	Cerrillos	light brown	scattered	5.17	MV
152	Cerrillos	dark brown	central	1.00	WV
153[e]	Cerrillos	light brown	scattered	3.00	WV
154[e]	Cerrillos	dark brown	central	4.17	MV
155	Cerrillos	light brown	central–peripheral	3.00	WV
156	Cerrillos	dark brown	central–peripheral	4.83	MV
157	Cerrillos	light brown	scattered	6.17	HV
158	Cerrillos	dark brown	central	5.51	HV
159[e]	Cerrillos	light brown	central–peripheral	ND	ND
160[e]	Cerrillos	dark brown	scattered circular	1.00	WV
161	Cerrillos	white	scattered	3.33	WV
162	Cerrillos	white	central–peripheral	3.33	WV
163	Cerrillos	light brown	scattered	2.33	WV
164	Cerrillos	dark brown	scattered	6.67	HV
165	Cerrillos	light brown	central	1.00	WV
166[d]	Cerrillos	white	scattered	2.00	WV
167[e]	R. de Lerma	dark brown	scattered	6.50	HV
168	R. de Lerma	white	central–peripheral	1.00	WV
169[e]	R. de Lerma	white	peripheral	2.00	WV
170[e]	R. de Lerma	white	central	3.50	MV
171[e]	R. de Lerma	light brown	central	3.67	MV
172[e]	R. de Lerma	light brown	peripheral	6.83	HV
173	R. de Lerma	light brown	peripheral	3.67	MV
174	R. de Lerma	white	peripheral	3.33	WV
175[e]	El Carril	light brown	peripheral	5.83	HV
176[e]	El Carril	light brown	peripheral	3.83	MV
177[e]	El Carril	white	scattered	1.33	WV
178[e]	El Carril	dark brown	peripheral	4.50	MV
179	El Carril	white	central	3.50	MV
180[de]	El Carril	light brown	central–peripheral	1.83	WV
181	El Carril	white	scattered	3.50	MV
182	R. de Lerma	light brown	central	2.33	WV
183[e]	R. de Lerma	light brown	peripheral	4.17	MV
184	R. de Lerma	light brown	scattered	6.00	HV
2951[e]	La Calderilla	light brown	central–peripheral	4.17	MV
2952[e]	La Calderilla	dark brown	central–peripheral	1.67	WV
2953[e]	La Calderilla	light brown	central	6.00	HV
2954[de]	La Calderilla	light brown	central	4.17	MV
2955	La Calderilla	light brown	central	ND	ND
2956	La Calderilla	dark brown	scattered	ND	ND
9061	La Silleta	light brown	central	6.17	HV
9062	La Silleta	light brown	scattered	ND	ND
9063[d]	La Silleta	light brown	central	4.67	MV

[a]R. de Lerma: Rosario de Lerma; [b]Disease severity index mean of web blight score based on a 1–9 scale; LSD (0.05): 2.26. Overall mean: 4.33; ND: not determined; [c]WV: weakly virulent (DSI $\leq$ 3); MV: moderately virulent (3 < DSI < 7); HV: highly virulent (DSI $\geq$ 7); [d]isolates that caused typical hypocotyl rot symptoms (1 < DSI $\leq$ 3); [e]isolate sequenced.

CA, USA) at 90 V in 1× 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).

Internal transcribed spacer (ITS) phylogenetic analysis

The rDNA-ITS region of 50 randomly selected isolates 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, 1× 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). 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. The products of the reaction were visualized as described above. The fragments were purified and subjected to sequencing in both directions using primers ITS1 and ITS4 on the 3500xL Genetic Analyzer sequencer (Applied Biosystems, Foster City, CA, USA) at the Biotechnology Institute of INTA (Castelar, Buenos Aires, Argentina). Forward and reverse sequences were assembled and aligned using BioEdit version 7.2.5 (Hall 1999). The isolates were identified to the species level by conducting Basic Local Alignment Search Tool (BLAST) searches with the GenBank sequence data.

The terminal units for the phylogenetic analysis were 50 isolates obtained in the present work and 48 control sequences retrieved from GenBank. *Ceratobasidium cereale* was used as outgroup. Alignment was performed using CLUSTAL W (Thompson et al. 1994) and adjusted by eye. Our dataset included 660 aligned nucleotide positions. jModelTest software v.2 (Guindon and Gascuel 2003; Darriba et al. 2012) was used to infer the most appropriate model of molecular evolution.

The general time reversible with rate variation among sites and a proportion of invariant sites (GTR + I + G) were selected as the best fit model of nucleotide substitution.

Bayesian phylogenetic analysis was performed using the 'metropolis-coupled Markov chain Monte Carlo' (MC3) algorithm implemented in MrBayes version 3.1.2 (Ronquist and Huelsenbeck 2003). Programme defaults were used for estimation of priors. Two independent analyses were run using a random starting tree over 10 000 000 generations sampling every 100 generations. Tree space was explored using one cold chain and three incrementally heated ones, with temperature set to 0.10. We assessed stationarity of the cold Markov chain for all MrBayes analyses through the standard deviation of the split frequencies. All posterior samples of a run prior to the burn-in point (at 25% of sampled topologies) were discarded. Remaining trees were used to obtain a 50% majority rule consensus tree with mean branch length estimates. Node support was assessed by posterior probability (Huelsenbeck and Ronquist 2001).

Pathogenicity characterization

Pathogenicity tests for each isolate 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). Wheat grains were transferred to a 5-day-old culture grown on PDA and incubated in darkness at 25 ± 2°C for 2 weeks. Twelve plants were inoculated by depositing wheat grains colonized with *R. 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, which were removed after 24 h, when 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 symptoms of the disease; 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 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) using InfoStat statistical software (Di Rienzo et al. 2014). Based on the DSI on foliar tissue, the isolates were classified into three virulence categories: weakly virulent (DSI ≤ 3), moderately virulent (3 < DSI < 7) and highly virulent (DSI ≥ 7). Re-isolations were made from the plants showing symptoms to confirm the pathogenic nature of the isolates.

Results

Morphological characterization

A total of 97 isolates were eventually used in this study, including 88 isolates from bean cultivars and

nine from wild bean populations (*Phaseolus vulgaris* var. *aborigineus*) collected from the Lerma Valley region in Salta province, north-western Argentina (Table 1).

All 97 isolates were identified as *R. solani* on the basis of cultural characteristics, and all of them presented multinucleate hyphal cells with an average of five nuclei per cell, varying from 2 to 9. The mycelium colour observed was white (20.6%), light brown (62.9%) or dark brown (16.5%).

Variability was observed in the pattern of sclerotia formation in the culture plates. Forty per cent of the isolates showed a central sclerotia formation, whereas 16.5% produced peripheral sclerotia, 20.6% produced scattered sclerotia and 22.7% showed central–peripheral sclerotia formation in plate (Table 1).

Molecular characterization

The DNA from each of the isolates was amplified using the specific primers for *R. solani* AG 2-2WB developed by Godoy-Lutz et al. (2008). All the PCR amplifications generated a 500 bp fragment in agreement with the amplification pattern reported for AG 2-2WB isolates (Godoy-Lutz et al. 2008).

Amplification of the ITS regions with ITS1/ITS4 primers yielded a DNA fragment of approximately 700 bp and uniform size among all isolates tested. A group of 50 randomly chosen PCR products were sequenced, and the ITS sequences were deposited in GenBank (KU361244-KU361293). Sequence analysis of the PCR products and comparison with NCBI available sequences showed that all the isolates belonged to AG 2-2WB with a 97–100% similarity.

Sequence polymorphism was observed within the ITS1 region. The phylogenetic tree showed that the isolates tested and the sequences retrieved from GenBank separated distinctly based on their AG (Fig. 1).

Pathogenicity assay

Most of the isolates were virulent to common bean and produced typical WB symptoms on foliar tissues (Table 1). Only seven isolates were non-pathogenic under the conditions of the test. Significant differences in aggressiveness were observed among isolates ($F = 4.28$, $P < 0.0001$) (Table 1). Disease severity index ranged from 1 to 9 with a mean value of 4.33 across all the isolates. On the basis of the disease reaction on foliar tissues, the isolates were grouped into three virulence categories as follows: weakly virulent (27), moderately virulent (34) and highly virulent (28) (Table 1). Isolates obtained from wild beans were represented in all the virulence categories. No correlation was observed between virulence and geographical origin, as isolates from the three virulence categories were present in all the fields sampled.

Only nine isolates (9.28%) were able to cause typical hypocotyl rot symptoms after 12 days of inoculation (Table 1). However, all of them were less aggressive on hypocotyls than on leaves, with low disease severity ratings ($1 < DSI \leq 3$). The rest of the isolates were not able to cause symptoms on hypocotyls or roots under the conditions of the test. All non-inoculated plants remained healthy.

Discussion

In the present study, 97 *R. solani* isolates were recovered from commercial bean cultivars (88) and wild beans (9) showing WB symptoms in the Lerma Valley region in Salta province, north-western Argentina. These isolates, characterized as AG 2-2WB based on the molecular analysis using specific primers, exhibited considerable variability in morphological characteristics and virulence.

Isolates of AG 2-2WB associated with bean WB were also reported in other common bean growing areas worldwide, including Honduras, Costa Rica, Dominican Republic and Ecuador (Godoy-Lutz et al. 2003, 2008; Mora-Umaña et al. 2013). Surprisingly, although AG 1-IE and AG 1-IF have been reported as the most widely distributed WB pathogens in bean fields in Latin America and the Caribbean (Godoy-Lutz et al. 2008; Gonzalez et al. 2012; Mora-Umaña et al. 2013), AG 1 was not detected in the isolates analysed. Godoy-Lutz et al. (2003, 2008) reported the occurrence of AG 2-2WB collected from wild beans showing WB symptoms in north-western Argentina. However, our results correspond to the first report of *R. solani* AG 2-2WB causing WB in commercial common bean fields in the country.

Sequence alignment and phylogenetic analysis of the rDNA-ITS region confirmed the identification of the isolates as AG 2-2WB, suggesting that specific primers and the ITS sequencing are powerful tools for *R. solani* AG identification and phylogenetic analysis, as previously reported (Kuninaga et al. 1997; Carling et al. 2002; Godoy-Lutz et al. 2008; Gonzalez et al. 2012; Mora-Umaña et al. 2013). The existence of polymorphisms within the AG group was evidenced, as a 100% match was observed only in a few sequences. This finding was in agreement with previous reports of variation at the ITS1 region of *R. solani* isolates within AGs (Justesen et al. 2003; Gonzalez et al. 2012). Low levels of variability in ITS sequences of AG1-IE and AG-1IF isolates from Latin America

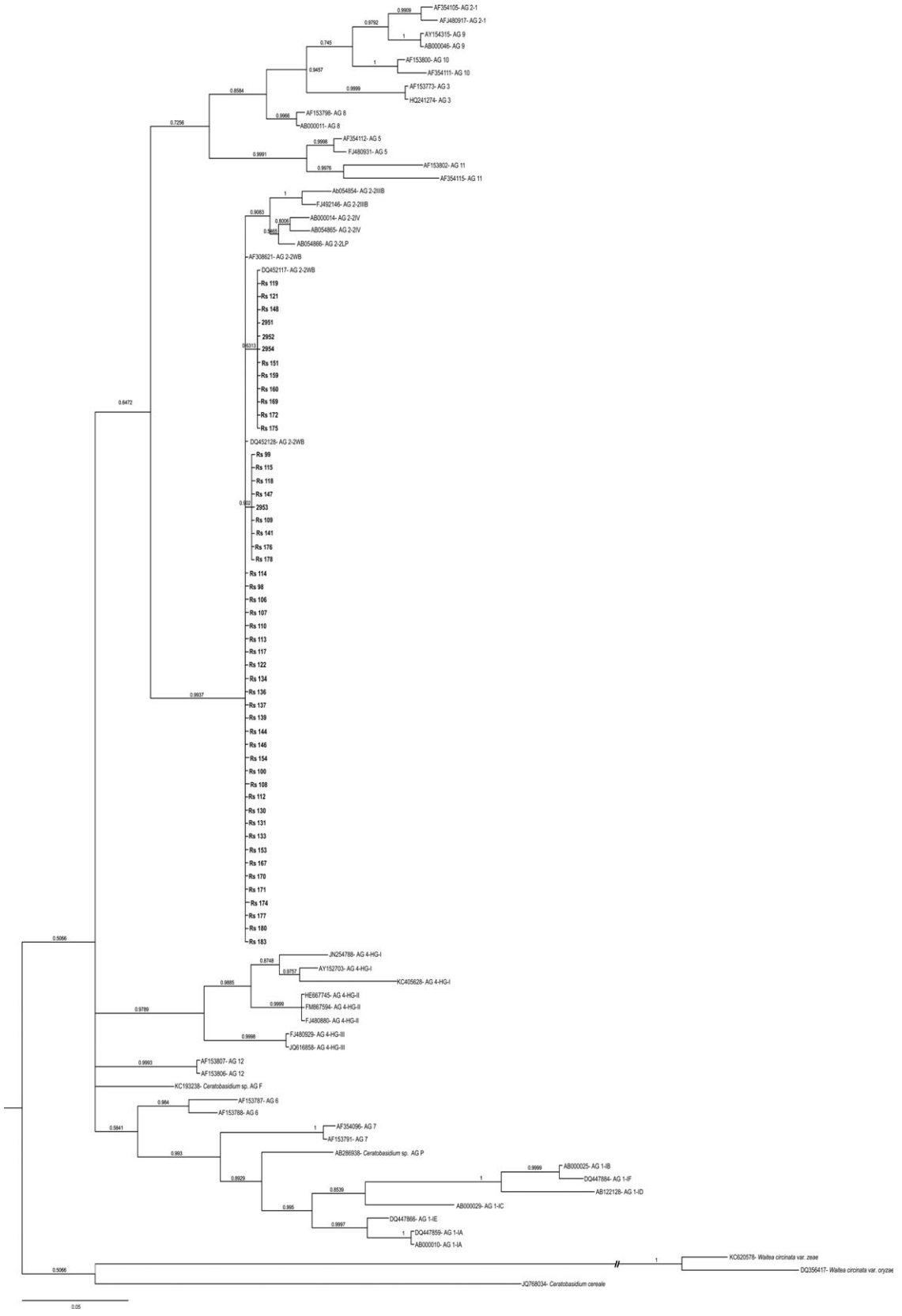
AF354105- AG 2-1
AF480917- AG 2-1
AY154315- AG 9
AB000046- AG 9
AF153800- AG 10
AF354111- AG 10
AF153773- AG 3
HQ241274- AG 3
AF153798- AG 8
AB000011- AG 8
AF354112- AG 5
FJ480931- AG 5
AF153802- AG 11
AF354115- AG 11
Ab054854- AG 2-2IIIB
FJ492146- AG 2-2IIIB
AB000014- AG 2-2IV
AB054865- AG 2-2IV
AB054866- AG 2-2LP
AF308621- AG 2-2WB
DQ452117- AG 2-2WB
Rs 119
Rs 121
Rs 148
2951
2952
2954
Rs 151
Rs 159
Rs 160
Rs 169
Rs 172
Rs 175
DQ452128- AG 2-2WB
Rs 99
Rs 115
Rs 118
Rs 147
2953
Rs 109
Rs 141
Rs 176
Rs 178
Rs 114
Rs 98
Rs 106
Rs 107
Rs 110
Rs 113
Rs 117
Rs 122
Rs 134
Rs 136
Rs 137
Rs 139
Rs 144
Rs 146
Rs 154
Rs 100
Rs 108
Rs 112
Rs 130
Rs 131
Rs 133
Rs 153
Rs 167
Rs 170
Rs 171
Rs 174
Rs 177
Rs 180
Rs 183
JN254788- AG 4-HG-I
AY152703- AG 4-HG-I
KC405628- AG 4-HG-I
HE667745- AG 4-HG-II
FM867594- AG 4-HG-II
FJ480680- AG 4-HG-II
FJ480929- AG 4-HG-III
JQ616858- AG 4-HG-III
AF153807- AG 12
AF153806- AG 12
KC193238- Ceratobasidium sp. AG F
AF153787- AG 6
AF153788- AG 6
AF354096- AG 7
AF153791- AG 7
AB286938- Ceratobasidium sp. AG P
AB000025- AG 1-IB
DQ447884- AG 1-IF
AB122128- AG 1-ID
AB000029- AG 1-IC
DQ447866- AG 1-IE
DQ447859- AG 1-IA
AB000010- AG 1-IA
KC620578- Waitea circinata var. zeae
DQ356417- Waitea circinata var. oryzae
JQ768034- Ceratobasidium cereale
0.05

Fig. 1 Phylogenetic tree based on rDNA-ITS sequences of 50 *Rhizoctonia solani* isolates recovered from commercial bean cultivars and wild beans showing WB symptoms in the Lerma Valley region in Salta, north-western Argentina, and 48 control sequences retrieved from GenBank. The tree was constructed using the GTR + I + G substitution model for Bayesian analysis. 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.

and the Caribbean have been reported by Godoy-Lutz et al. (2008). However, Gonzalez et al. (2012) included a greater number of isolates per site and observed intragroup variation when analysing the rDNA-ITS sequence. Moreover, their results were in agreement with the analysis based on URP and ISSR molecular markers and mycelial compatibility (Gonzalez et al. 2012).

In the pathogenicity test, most of the isolates were able to cause disease symptoms on leaves and only six isolates were aggressive on hypocotyls, suggesting a relation between the source of the isolate (leaf lesions) and the type of disease caused. The present study supports previous observations, which stated that WB isolates added to soil were less pathogenic than those obtained from damping-off seedlings or root rots (Godoy-Lutz et al. 1996). These findings are also in agreement with studies performed with *R. solani* isolates from the same geographical region on another host, like tobacco (Mercado-Cárdenas et al. 2015).

A great variability in virulence was observed among the isolates analysed, supporting the idea of intragroup variation. Similar results were reported by Meinhardt et al. (2002), who observed a great virulence and genetic variability among AG 4 HG-I isolates from Brazil. No information is currently available regarding the population genetic structure of *R. solani* AG 2-2WB affecting common bean in Latin America or the role of the sexual stage of the pathogen in the disease epidemiology. A deeper analysis on population genetics based on codominant markers would help to elucidate if the population structure of the pathogen is derived from an outbreeding, clonal or mixed model as described for *R. solani* AG 1-IA (Linde et al. 2005; Ciampi et al. 2008; Gonzalez-Vera et al. 2010), AG 3 (Ceresini et al. 2007) and AG 4 (Haratian et al. 2013) in other areas. 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.

The information generated in the present study provides initial data on the population variability of the WB pathogen in north-western Argentina and represents a valuable contribution to regional breeding programmes aimed to obtain cultivars with durable resistance. Moreover, the pathogen variability observed is relevant to the disease management in order to develop successfully integrated control programmes and minimize yield losses due to WB. To elucidate the population genetic structure of the pathogen, surveys based on molecular markers are in progress.

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