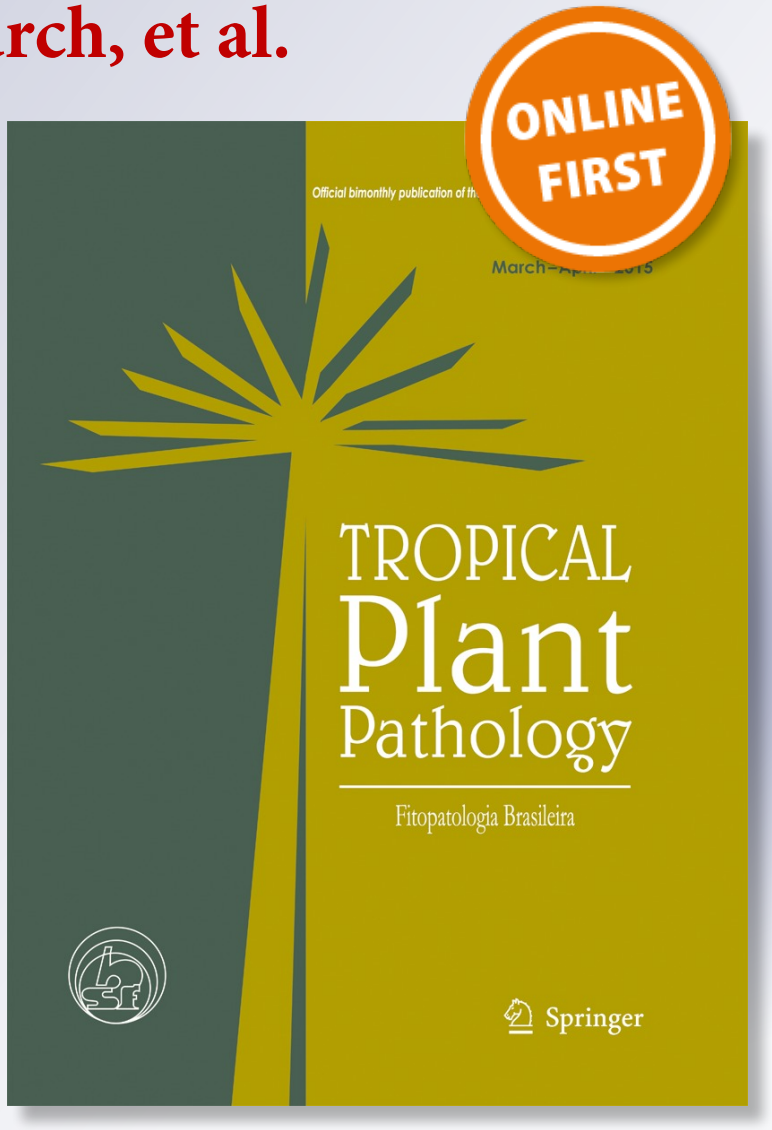
Molecular identification and pathogenicity of Rhizoctonia *spp. from tobacco growing areas in northwestern Argentina*

Guadalupe E. Mercado Cárdenas, Marta Z. Galván, Viviana A. Barrera, Marcela S. Rodriguero, Marcelo A. Carmona, Guillermo J. March, et al.

Tropical Plant Pathology

e-ISSN 1983-2052

Trop. plant pathol.
DOI 10.1007/s40858-015-0035-7



Springer

Trop. plant pathol.
DOI 10.1007/s40858-015-0035-7

Molecular identification and pathogenicity of *Rhizoctonia* spp. from tobacco growing areas in northwestern Argentina

Guadalupe E. Mercado Cárdenas[1] · Marta Z. Galván[1,2] · Viviana A. Barrera[3] · Marcela S. Rodriguero[4] · Marcelo A. Carmona[5] · Guillermo J. March[6] · Ana C. Ramallo[7] · H. David Shew[8]

Received: 7 October 2014 / Accepted: 27 January 2015

Abstract In Argentina, more than 60 % of the tobacco crops are grown in the northwestern part of the country and where *Rhizoctonia solani* leads to a reduction in crop yield and quality. In this study, 35 isolates of *Rhizoctonia* were obtained from 32 tobacco fields in northwestern Argentina and characterized by both morphological and molecular approaches. Based on the variability in the ITS region, isolates were identified as *R. solani* (80 %), *Waitea circinata* var. *zeae* (*Rhizoctonia zeae*) (8 %) and binucleate *Rhizoctonia* (8 %). Most isolates of *R. solani* belonged to the anastomosis groups (AGs) AG 4 HG-I (44 %), AG 2-1 (41 %) and AG 4 HG-III (13 %). Isolates of binucleate *Rhizoctonia* belonged to AG-F and AG-P of *Ceratobasidium* sp. Morphological variability was higher within isolates of AG 2-1 and AG 4 HG-III than within those of AG 4 HG-I. Aggressiveness of the isolates towards tobacco seedlings was assessed in the greenhouse. Isolates of AG 2-1 were the most aggressive on leaves, causing target spot, whereas isolates of AG 4 HG-I were the most aggressive on stems and roots, causing damping-off.

Keywords *Nicotiana tabacum* · ITS · Anastomosis group (AG) · Vegetative compatibility

Section Editor: Adalberto Café-Filho

✉ Guadalupe E. Mercado Cárdenas
mercado.guadalupe@inta.gob.ar

[1] Instituto Nacional de Tecnología Agropecuaria (INTA) EEA Salta, Ruta Nac. 68 Km 172 (4403) Cerrillos, Salta, Argentina

[2] Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), CCT-Salta. Av. Bolivia 5150 (4400), Salta, Argentina

[3] IMYZA, INTA Castelar, Buenos Aires, Argentina

[4] Laboratorio de Genética Evolutiva, Departamento de Ecología, Genética y Evolución, UBA, Buenos Aires, Argentina

[5] FAUBA, Buenos Aires, Argentina

[6] IPAVE, INTA, Córdoba, Argentina

[7] UNT, Tucumán, Argentina

[8] Department of Plant Pathology, North Carolina State University, Raleigh, NC 27695, USA

Introduction

Tobacco (*Nicotiana tabacum* L.) is commercially produced in more than 128 countries worldwide. Argentina is among the ten main producers of unmanufactured tobacco, with over 60 % of the tobacco grown in northwestern region of the country (FAO 2012). The crop is grown mainly in the provinces of Salta and Jujuy, where it achieves a mean yield of 2000 kg/ha. The main constraints for tobacco production in Argentina are root and stem diseases caused by the soilborne pathogens *Rhizoctonia solani*, *Ralstonia solanacearum*, *Fusarium oxysporum* f. sp. *nicotianae*, and *Phytophthora nicotianae*.

Among the *Rhizoctonia* species pathogenic to tobacco, multinucleate species such as the teleomorphs *Thanatephorus cucumeris* (*Rhizoctonia solani*) and *Waitea circinata* var. *zeae* (*Rhizoctonia zeae*), and binucleate species such as *Ceratobasidium* spp. have been reported (Lucas 1975; Nicoletti and Lahoz 1995; Shew and Melton 1995; Garcia et al. 2009; Gurkanli and Ozkoc 2011). In tobacco, these pathogens cause several symptoms including target spot, stem rot and damping-off in transplants and target spot and sore shin in field plants, resulting in crop losses of up to 15 % (Lucas 1975; Shew and Lucas 1991; Csinos and Stephenson 1999; Tarantino 2007; Gonzalez et al. 2011). Under wet conditions, especially rainy weather, target spot epidemics may lead to severe losses. However, these conditions are not frequent in

Published online: 11 July 2015

tobacco growing areas worldwide (Sherwood 1969; Shew and Melton 1995; Gurkanli et al. 2009).

Within *R. solani*, identification is based on the mycelial compatibility/incompatibility between isolates, which enables to assign them to anastomosis groups (AGs) (Sneh et al. 1991; Carling 1996). Currently, 13 AGs have been reported (AG 1–13), with many of the AGs further divided into subgroups based on DNA sequencing (Laroche et al. 1992; Johnk and Jones 1993; Liu and Sinclair 1993; Liu et al. 1993; Tu et al. 1996; Carling 1996; Sharon et al. 2008; González García 2008). Isolates of AG 1, AG 2-2, AG 4 and AG 5 cause damping-off and sore shin of tobacco (Gutierrez et al. 1997; Nicoletti and Lahoz 1995), whereas isolates of AG 3 cause target spot (Shew and Melton 1995; Zhao et al. 2013). Recently, binucleate (BN) *Rhizoctonia* AG-A, AG-F, AG-G, AG-P, AG-R have also been reported as the cause of damping-off in tobacco (Masuka 1998; Garcia et al. 2009; Gurkanli and Ozkoc 2011).

Although the anastomosis procedure of identification and classification is still used, it requires availability of tester isolates (Sharon et al. 2006; Garcia et al. 2009). In recent years, the classical AG-grouping has been confirmed by the use of DNA-based techniques. Sequence analysis of the 18S, 28S and 5.8S transcriptional units and the ITS regions of the rDNA genes has been used to infer taxonomic and phylogenetic relationships among different AGs of *R. solani* and *Rhizoctonia* spp., facilitating identification of this species complex (Sharon et al. 2006, 2008). Moreover, ITS sequences have been proposed as a DNA barcode for some fungi (Schoch et al. 2012).

Few studies of the populations of *Rhizoctonia* spp. that cause tobacco diseases in different geographic regions have been reported, and, to our knowledge, there are no studies on species identification conducted with Argentinean populations. Thus, the aims of this work were to identify the species of *Rhizoctonia* associated with tobacco diseases in northwestern Argentina and to assess their aggressiveness towards tobacco under greenhouse conditions.

Materials and methods

Survey and fungal isolation

Surveys were conducted in northwestern Argentina tobacco fields during the 2008–2011 growing seasons. Plants exhibiting damping-off, sore shin or target spot symptoms were collected from 32 different tobacco fields (Table 1). A total of 39 isolates were obtained: 25 from symptomatic tobacco plants, 10 from the soil (described below), and four from host used in crop rotation exhibiting symptoms of disease caused by *Rhizoctonia* (Table 1). Isolation from plant tissues was made on potato dextrose agar medium (PDA; Britania S.A.) acidified to pH 5 with 10 % lactic acid and incubated at 25±2 °C in darkness for 2 to 3 days. Isolation from soil was made 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 based on morphological features and DNA characterization (Sneh et al. 1991).

Morphological characterization

Morphological characteristics of the isolates were described based on the observation of cultures grown on PDA at 10, 20, 30 and 40 °C in the dark for 20 d (Table 2). Isolates were assigned to morphological groups based on the color and growth of the mycelium and the type of sclerotia produced (color and whether they were produced either in or on the agar medium). A rapid nuclear-staining procedure using acridine orange (Sneh et al. 1991) was performed to determine the number of nuclei in each hyphal cell. The stained cells were observed by fluorescence microscopy. All isolates studied were deposited in the collection of the "Laboratorio de Sanidad Vegetal" INTA-EEA-Salta Microbial Collection, Argentina.

ITS-rDNA gene sequencing

For molecular characterization, genomic DNA was extracted from 250 mg of hyphal tissue using a CTAB protocol (Stenglein and Balatti 2006). The ribosomal DNA internal transcribed spacer region (ITS-rDNA) 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.) 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 in a thermocycler (Eppendorf Master Cycler) programmed with an initial denaturing step at 94 °C for 1 min, followed by 30 cycles at 94 °C for 15 s, annealing at 58 °C for 15 s and 72 °C for 15 s, with a final extension step at 72 °C for 7 min. The products of the reaction were visualized in 2 % agarose gels containing 0.2 μg/μl ethidium bromide and purified using

Table 1 Anastomosis group (AG), isolate code, source, origin, year of collection, nuclear status, disease severity index (DSI) and GenBank accession number of 39 isolates of *Rhizoctonia* spp. collected in different fields of northwestern Argentina during the 2008–2011 growing seasons

AG/ Subgroup[1]	Isolate	Symptoms/ Source/Host[2]	Origin (location/province)	Field N°	Year[3]	Nuclear status[4]	Severity[5]	GenBank N°
Rhizoctonia solani								
AG 2-1	Rs66	TS/leaves/*Nt*	Cerrillos/Salta	1	2010	MN	3.1	JQ616852
AG 2-1	Rs67	TS/leaves/*Nt*	Cerrillos/Salta	1	2010	MN	0.0	JQ616853
AG 2-1	Rs59	TS/leaves/*Nt*	La Silleta/Salta	2	2010	MN	4.2	JQ616867
AG 2-1	Rs59b	TS/leaves/*Nt*	La Silleta/Salta	3	2010	MN	4.2	JQ616874
AG 2-1	Rs68	TS/leaves/*Nt*	Cerrillos/Salta	4	2010	MN	3.1	JQ616870
AG 2-1	Rs69	TS/leaves/*Nt*	Cerrillos/Salta	5	2010	MN	1.1	JQ616871
AG 2-1	Rs70	TS/leaves/*Nt*	Cerrillos/Salta	6	2010	MN	1.2	JQ616872
AG 2-1	Rs59c	TS/leaves/*Nt*	La Silleta/Salta	3	2010	MN	4.2	–
AG 2-1	Rs25	SS/root/*Nt*	La Merced/Salta	7	2008	MN	0.1	JQ616864
AG 2-1	Rs25b	SS/root/*Nt*	La Merced/Salta	8	2008	MN	1.1	JQ616849
AG 2-1	Rs36	SS/root/*Nt*	La Merced/Salta	9	2009	MN	0.0	JQ616865
AG 2-1	Rs36b	SS/root/*Nt*	La Merced/Salta	10	2009	MN	0.1	JQ616854
AG 2-1	Rs58	DO/root/*Nt*	La Silleta/Salta	11	2010	MN	2.4	JQ616866
AG 4 HG-I	Rs46	SS/root/*Nt*	R. de Lerma/Salta	12	2010	MN	0.1	JQ616862
AG 4 HG-I	Rs46b	SS/root/*Nt*	R. de Lerma/Salta	13	2010	MN	0.1	JQ616868
AG 4 HG-I	Rs47	SS/root/*Nt*	R. de Lerma/Salta	14	2010	MN	1.4	JQ616860
AG 4 HG-I	Rs47b	SS/root/*Nt*	R. de Lerma/Salta	15	2010	MN	1.4	JQ616855
AG 4 HG-I	Rs79	SS/root/*Nt*	El Carmen/Jujuy	16	2011	MN	1.0	JQ616856
AG 4 HG-I	Rs80	SS/root/*Nt*	El Carmen/Jujuy	17	2011	MN	0.0	JQ616857
AG 4 HG-I	Rs44	SS/root/*Nt*	R. de Lerma/Salta	18	2009	MN	0.4	–
AG 4 HG-I	Rs60	-/soil/-	Cerrillos/Salta	19	2009	MN	1.0	JQ616850
AG 4 HG-I	Rs61	-/soil/-	Cerrillos/Salta	20	2009	MN	1.0	JQ616848
AG 4 HG-I	Rs63	-/soil/-	Cerrillos/Salta	21	2009	MN	1.1	JQ616851
AG 4 HG-I	Rs12	-/seed/*Ah*	Las Lajitas/Salta		2008	MN	0.0	JQ616861
AG 4 HG-I	Rs49	RR/root/*Ca*	Cerrillos/Salta		2011	MN	1.2	JQ616863
AG 4 HG-I	Rs53b	SS/root/*Nt*	Cerrillos/Salta	22	2009	MN	2.3	–
AG 4 HG-I	Rs73	SS/root/*Nt*	R. de Lerma/Salta	23	2011	MN	0.1	–
AG 4 HG-III	Rs74	DO/root/*Nt*	C. Moldes/Salta	24	2011	MN	0.1	JQ616869
AG 4 HG-III	Rs75	DO/root/*Nt*	C. Moldes/Salta	25	2011	MN	0.0	JQ616859
AG 4 HG-III	Rs81	RR/root/*S*	Guachipas/Salta		2011	MN	1.0	JQ616858
AG 4 HG-III	Rs72	-/soil/-	R. de Lerma/Salta	26	2010	MN	1.1	JQ616873
AG 8	Rs77	SS/root/*Nt*	Ovejería/Jujuy	27	2011	MN	0.0	–
Waitea circinata var. zeae								
–	Rs22a	-/soil/-	Los Lapachos/Jujuy	28	2008	MN	0.0	–
–	Rs22b	-/soil/-	Los Lapachos/Jujuy	28	2008	MN	0.0	–
–	Rs35	-/soil/-	Los Lapachos/Jujuy	29	2009	MN	0.0	–
Rhizoctonia sp.								
–	Rs65	-/soil/-	Cerrillos/Salta	30	2010	MN	0.0	–
Ceratobasidium sp.								
AG-F	Rs71	-/soil/-	R. de Lerma/Salta	31	2010	BN	2.0	–
AG-P	Rs71b	-/soil/-	R. de Lerma/Salta	32	2010	BN	2.0	–
AG-P	Rs56	-/seed/*Pv*	Cerrillos/Salta		2010	BN	1.0	–

[1] *AG* Anastomosis group determined by rDNA-ITS sequences

[2] *TS* target spot, SS sore shin, DO: damping-off, *RR* root rot, *Nt Nicotiana tabacum*, *Ah Arachis hypogea*, *Ca Cicer arietinum*, *S Sorghum* sp, *Pv*: *Phaseolus vulgaris*

[3] Year of collection

[4] *MN* Multinucleate, *BN* Binucleate

[5] 0–4 scale for target spot and damping-off. Disease severity indexes were assessed on tobacco cv. "K326" seedlings

a purification kit (QIAquick PCR Purification Kit, Qiagen Inc.). The purified products were subjected to sequencing in both directions using primers ITS1 and ITS4 in an ABI 3130XL DNA sequencer (Applied Biosystems).

Pathogenicity assay

Pathogenicity tests for each isolate were performed using tobacco plants (cv. K326) grown for 8 weeks 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 twice in the autoclave (120 °C for 30 min), on two successive days. Wheat grains were transferred to a 5-day-old culture grown on PDA and incubated in darkness at 25± 2 °C for 1 week. Ten plants were inoculated by depositing wheat grains colonized with *Rhizoctonia* spp. 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 and misted and covered with polyethylene bags that were removed after 2 days, when plants were moved to a glasshouse. Ten days after inoculation, the disease severity index (DSI) was rated for leaves and roots separately, using the following 0–4 scales, for target spot: 0=no infection, 1=1–25 % of the foliar area infected, 2=26–50 % of the foliar area infected, 3=51–75 % of the foliar area infected and 4=76–100 % of the foliar area infected; for damping-off: 0, no infection, 1=1–25 % of the root area infected, 2=26–50 % of the root area infected, 3=51–75 % of the root area infected and 4=76–100 % of the root area infected. Data were analyzed statistically using the Kruskal-Wallis test (p=0.005) to compare the DSI among and within AGs. The morphological characteristics of the pathogen re-isolated from symptomatic plants were consistent with *R. solani*.

Data analyses

Sequences of each isolate were edited using Bioedit (Hall 1999) and deposited in GenBank (see accession numbers in Table 1). The dataset included 39 isolates obtained in the present work and 41 control sequences retrieved from GenBank selected to represent different AGs. Isolates from other hosts (bean, chickpea, sorghum and peanut) were included in the analysis. Multiple alignments were performed using ClustalW (Thompson et al. 1994) and adjusted manually. Ambiguous regions were deleted. The complete dataset included 871 aligned nucleotide positions and 80 taxa. Cluster analysis was performed using the neighbor joining (NJ) method implemented in PAUP* v. 4.0 and visualized with TreeView version 1.6.6 (Page 1996; Swofford 2002). Group support was assessed through 1000 bootstrap replicates for both analyses.

Table 2 Morphological groups of *Rhizoctonia* spp. isolates based on mycelial and sclerotial characteristics

Morphological Group	Species/AG group[1] (isolates)	Mycelial Color	Mycelial Growth	Sclerotial Color	Sclerotial Position
MG-1	*R. solani*/AG 2-1 (Rs59, Rs59b, Rs59c, Rs58, Rs36b, Rs36, Rs25, Rs25b) *R. solani*/AG 4 HG-III (Rs81, Rs72) *R. solani*/AG 8 (Rs77) *Ceratobasidium* sp./AG-F (Rs71) *Ceratobasidium* sp./AG-P (Rs71b, Rs56)	Brown	Abundant	brown	Aerial
MG-2	*R. solani*/AG 2-1 (Rs70, Rs69, Rs68, Rs67, Rs66) *R. solani*/AG 4 HG-I (Rs80, Rs79, Rs73, Rs63, Rs61, Rs60, Rs49, Rs47, Rs47b, Rs46, Rs46b, Rs44, Rs12, Rs53b) *R. solani*/AG 4 HG-III (Rs75, Rs74)	Light brown	Poor	brown	Immersed and aerial
MG-3	*W. circinata* var. *zeae* (Rs22a, Rs22b)	Light orange	Abundant	Orange	Immersed and aerial
MG-4	*W. circinata* var. *zeae* (Rs35) *Rhizoctonia* sp. (Rs65)	Light brown	Poor	–	–

[1] *AG* Anastomosis group determined by rDNA-ITS sequences

Results

Morphological characterization

Isolations from diseased tobacco, soil, and rotation crops resulted in recovery of 39 isolates of *Rhizoctonia*; 36 isolates were multinucleate and 3 were binucleate (Table 1). The number of nuclei per cell in the multinucleate isolates varied from 2–8. All cells of the binucleate *Rhizoctonia* isolates contained only 2 nuclei per cell. Four morphological groups (MGs) were described based on isolate phenotypes (Table 2). All phenotypic characteristics were consistent with previous morphological reports for *Rhizoctonia* (Sneh et al. 1991). Mycelium was initially white and changed to light brown, brown or light orange after 48–72 h. After 2 weeks of incubation at 25±2 °C, all isolates produced brown or orange sclerotia. Sclerotia were produced on aerial hyphae or immersed in the medium. MG 1 and 2 included all the *R. solani* isolates analyzed.

ITS-rDNA phylogeny

Genomic DNA of the 39 *Rhizoctonia* spp. isolates was used as template for the amplification of the ITS1, 5.8 rDNA and ITS2 regions. The isolates tested yielded a 700-bp product. Thirty of the isolates analyzed were placed in a major cluster supported by a bootstrap value of 100 % and identified as *R. solani*, while the rest of the isolates corresponded to 4 *Rhizoctonia* spp. (Fig. 1). These species included *Waitea circinata* var. *zeae* (*Rhizoctonia zeae*) and the binucleate *Rhizoctonia* species (AG-F and AG-P). One isolate (Rs65) recovered from the soil showed high similarity (99 %) with a *Rhizoctonia* sp. (KC176298) reported as saprophytic (Thorn et al. 1996). According to the pathogenicity tests, this isolate was not virulent on the tobacco variety tested (Table 1). The *R. solani* isolates included 13 isolates of AG 2-1, 14 of AG 4 HG-I, 4 of AG 4 HG-III, and 1 of AG 8, with bootstrap values above 80 %.

Pathogenicity to foliar and root tissues

Seventy-one percent of the isolates caused disease on tobacco seedlings. Twenty-two isolates (56 %) caused target spot symptoms, whereas 22 isolates caused damping-off symptoms (Table 1). Seven of the isolates caused only target spot, and 7 other isolates caused only damping-off. All non-inoculated plants remained healthy. Typical symptoms of target spot were developed within 2 days after inoculation whereas typical symptoms of damping-off or stem rot were developed within 5 days. Severity ratings ranged from 0–4 for both diseases across all isolates. Eleven isolates were non-pathogenic under the conditions of the test. The source of the isolate was related to the type of disease observed in the inoculation trials. Most of the isolates obtained from leaf lesions (88 %) were highly aggressive on leaves, whereas isolates obtained from roots and soil caused little disease on leaf tissues, with disease severity ratings≤2. Binucleate isolates caused low levels of disease on leaves (Table 1). Isolates within each AG differed in their aggressiveness on leaves and stems (Fig. 2). For example, isolates of AG 4 were significantly less aggressive than those of AG 2-1 for target spot (p <0.0001) (Fig. 2), whereas those of AG 4 were the most aggressive isolates on stems (p=0.05).

Discussion

In the present study isolates of *Rhizoctonia* were recovered from tobacco plants and fields in northwestern Argentina (Salta and Jujuy provinces). Isolates were characterized as *Rhizoctonia solani* (82 %) and *Rhizoctonia* spp. (18 %) based on the molecular characterization of the ITS region.

Of the 32 isolates that were identified as *Rhizoctonia solani*, eleven were isolated from tobacco seedlings and fourteen from field plants, indicating that this pathogen is distributed both in seedlings and fields, as reported in other areas worldwide (Gonzalez et al. 2011). AG 4 HG-I was the prevalent group (44 %), followed by AG 2-1 (41 %), AG 4 HG-III (13 %) and AG 8 (3.1 %). Multiple AGs of *R. solani* are pathogenic on tobacco (Shew and Melton 1995; Nicoletti and Lahoz 1995; Gutierrez et al. 1997; Nicoletti et al. 1999; Zhao et al. 2013). AG 1, AG 2-1, AG 2-2 and AG 4 cause root rot, stem rot or sore shin, and damping-off, while AG 2-1 and AG 3 cause target spot on leaves of tobacco (Shew and Melton 1995; Gutierrez et al. 1997; Nicoletti et al. 1999; Mercado Cárdenas et al. 2012; Zhao et al. 2013). In our study the *R. solani* isolates identified as AG 2-1 were obtained from plants with damping-off, target spot and sore shin symptoms. On the other hand, AG 4 HG-I and AG 4 HG-III isolates were associated with sore shin and damping-off symptoms, respectively. Surprisingly, although AG 3 has been reported as the target spot pathogen widely distributed in tobacco growing areas worldwide, including Southern Brazil and Southern USA (Shew and Milton 1995; Ceresini et al. 2002; Gonzalez et al. 2011), AG 3 was not detected in the isolates analyzed. We recovered 1 isolate of AG 8 from a diseased tobacco plant, but this isolate was non-pathogenic in our inoculation trials.

The predominance of AG 4 HG-I and AG 2-1 isolates was also observed by Gurkanli et al. (2009) and Bacharis et al. (2010) in tobacco seedlings showing symptoms of damping-off in Turkey and Greece. In this study, isolates obtained from plants with damping-off symptoms were identified as AG 4 HG-III and AG 2-1. However, a more thorough study increasing the number of samples would be necessary to obtain comparable results.

Two BN *Rhizoctonia* isolates from soil and 1 from bean seeds were identified as AG-F and AG-P. All of them caused target spot symptoms on tobacco in our inoculation trials. There are reports of BN *Rhizoctonia* causing damping-off

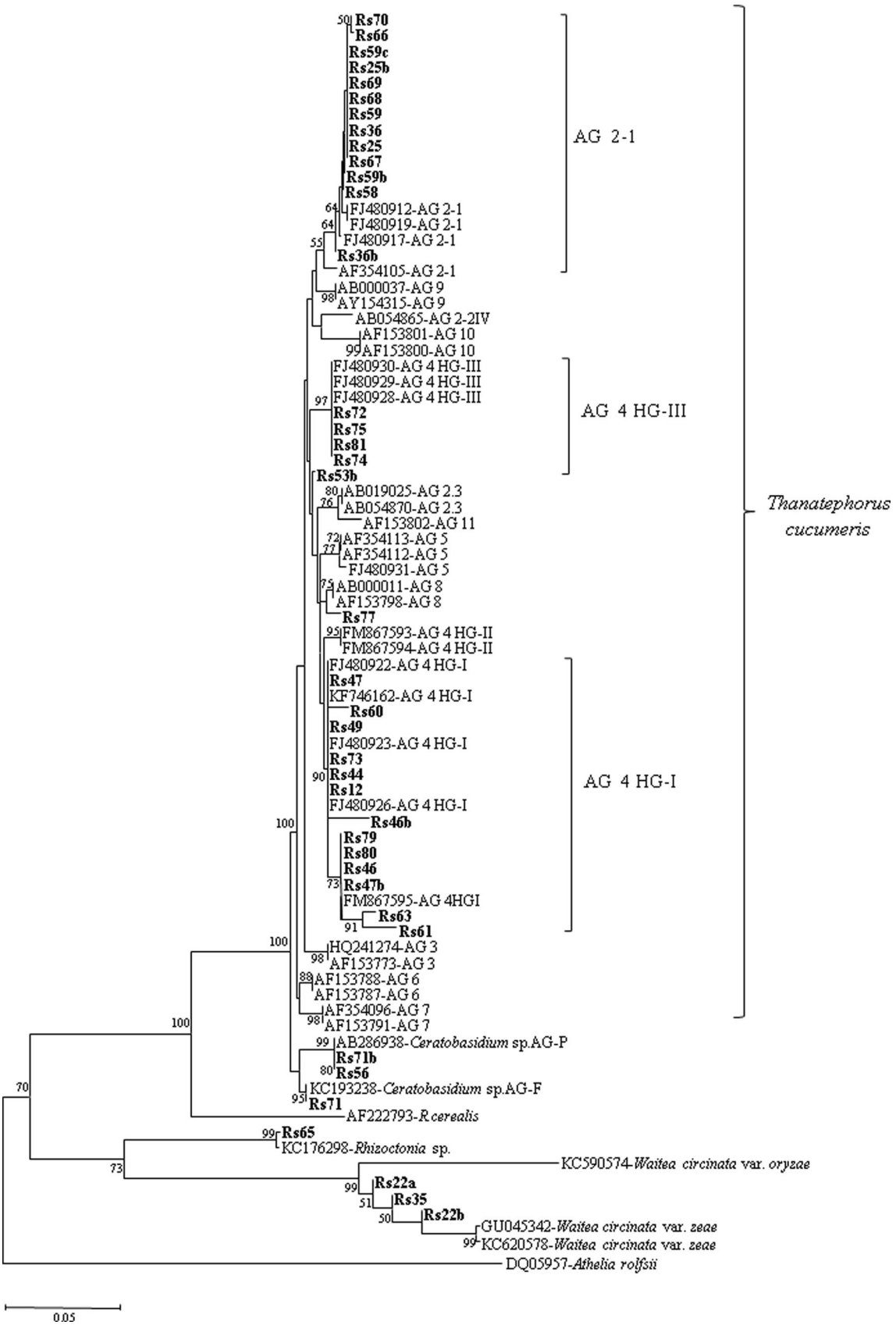


Fig. 1 Neighbor-joining tree based on rDNA-ITS sequences of *Rhizoctonia* spp. isolates and corresponding sequences from GenBank. *Athelia rolfsii* was used as an outgroup. Only bootstrap values above 50 are reported

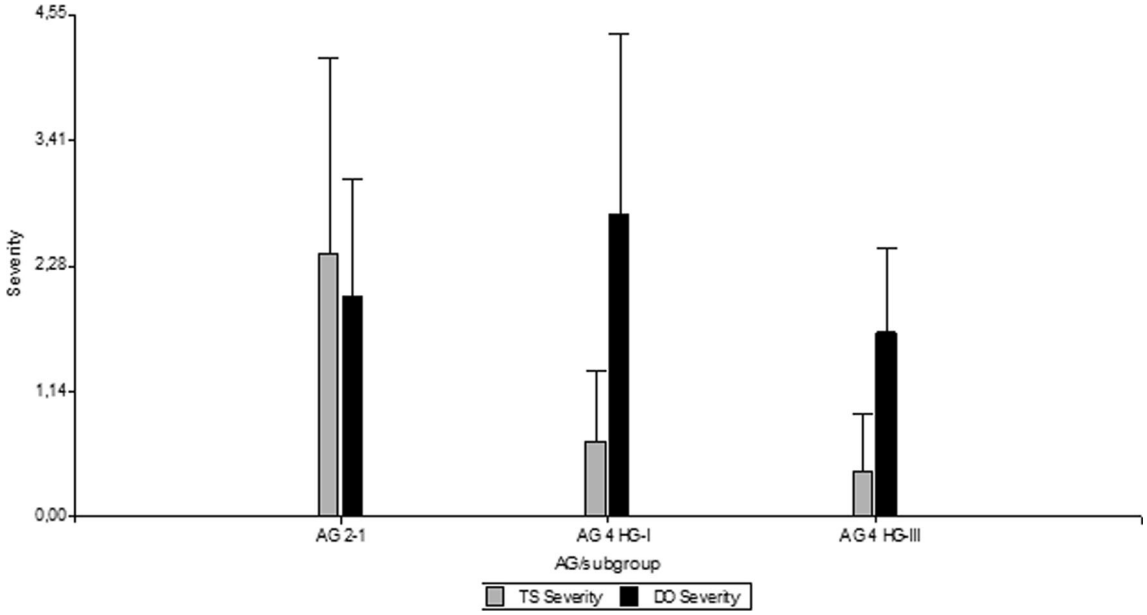


Fig. 2 Disease severity index (DSI) of target spot and damping-off of 3 *Rhizoctonia solani* AGs (AG 2-1, AG 4 HG-I and AG 4 HG-III)

and root rot in tobacco (Garcia et al. 2009; González García 2008; Gurkanli and Ozkoc 2011; Masuka 1998), but not leaf spot as we observed.

All the *R. solani* isolates were included in only 2 of our 4 morphological groups, MG-1 and MG-2. They all fit well with the *R. solani* description criteria of Sneh et al. (1991), including the number of nuclei per cell, the type of sclerotium produced, and the brown pigmentation of the mycelium with age. Morphological variability was higher within AG 2-1 and AG 4 HG-III than within isolates of AG 4 HG-I.

A significant relationship was observed between the AG and the type of disease symptoms. In the pathogenicity tests, *R. solani* AG 2-1 and AG 4 HG-I isolates caused the highest severity rating, with the highest DSI for target spot and damping-off, respectively. These results support previous observations (Gurkanli et al. 2009; Bacharis et al. 2010). *R. solani* AG 4 HG-I has been reported to be more aggressive than AG 4 HG-II and AG 2-1, causing damping-off symptoms on tobacco seedlings (Gurkanli et al. 2009; Bacharis et al. 2010).

R. solani AG 4 HG-I and AG 4 HG-III caused target spot symptoms on tobacco under greenhouse conditions. However, none of these isolates were obtained from tobacco plants showing leaf spot symptoms. There is not enough evidence of *R. solani* AG 4 causing target spot under natural conditions. Only Shew et al. (1995) cited that a few isolates of AG 4 were obtained from leaf spots in North Carolina (USA) during the 1984 and 1989 epidemics.

AG 2-1 isolates caused damping-off symptoms in tobacco transplants and sore shin in older field plants as observed in other studies (Bacharis et al. 2010; Gutierrez et al. 1997; Nicoletti and Lahoz 1995). We have recently reported that *R. solani* AG 2-1 is associated with target spot on tobacco seedlings produced under float systems, but made no field observations (Mercado Cárdenas et al. 2012).

The information generated in this study is relevant to the management of tobacco disease in the northern region of Argentina. Since *R. solani* has been isolated from seedlings which represent a potential source of inoculum, it is important to improve the production of healthy seedlings to reduce the inoculum in fields and consequently the epidemics. Moreover, the correct identification of pathogens causing disease is important considering that AGs vary in their sensitivity to fungicides (Jones and Belmar 1989; Pascual et al. 2000). Also, AG differentiation is important in intercropping or crop rotation decisions because AG 2-1, AG 4 HG-I and AG 4 HG-III can be pathogenic to rotation crops such as bean and maize (Godoy-Lutz et al., 2003, 2008; Li et al. 1998; Tu et al. 1996). In the present study, only 4 isolates were obtained from other rotation crops and showed low levels of aggressiveness in the pathogenicity tests. The analysis of a larger number of isolates obtained from rotation crops would enable the identification of the best crop rotation to reduce the incidence of *Rhizoctonia* diseases in tobacco fields in northwestern Argentina.

Acknowledgments We thank C. Sanchez for statistical advice. Thanks are also given to Instituto Nacional de Tecnología Agropecuaria for providing a doctorate fellowship for the first author and support by means of research grants (PNIN082511 and PRSalJu09).

References

Alfenas A, Valverde E, Gomeide E, Ferreira A, Murilo F, Pereira F, Rogeiro J, Grassi L, Maffia L, Pereira O, Alfenas P, Gonçalves R,

Gonçalves R, D'Arc R, Dos Santos W (2007) Métodos em Fitopatologia. Brasil. Ed. Universidade de Visçosa, Visçosa

Bacharis C, Gouziotis A, Kalogeropoulou P, Koutita O, Tzavella-Klonari K, Karaoglanidis G (2010) Characterization of *Rhizoctonia* spp. isolates associated with damping-off disease in cotton and tobacco seedlings in Greece. Plant Dis 94:1314–1322

Carling D (1996) Grouping in *Rhizoctonia solani* by hyphal anastomosis reaction. In: Sneh B, Jabaji-Hare S, Neate S, Dijst G (eds) *Rhizoctonia* Species: taxonomy, molecular biology, ecology, pathology and disease control. Kluwer Academic Publishers, Dordrecht, pp 37–47

Ceresini PC, Shew HD, Vilgalys RJ, Cubeta MA (2002) Genetic diversity of *Rhizoctonia solani* AG-3 from potato and tobacco in North Carolina. Mycologia 94:437–449

Csinos AS, Stephenson MG (1999) Evaluation of fungicides and tobacco cultivar resistance to *Rhizoctonia solani* incited target spot, damping-off and sore shin. Crop Prot 18:373–377

Food and Agriculture Organization-FAO (2012) Production Crops 2012. http://www.faostat.fao.org/site/567/default.aspx#ancor.html. Accessed 3 Oct 2014

Garcia MG, Ramos ER, Chacon OC, Bocourt YP, Ochoa RR (2009) First report of binucleate *Rhizoctonia* causing damping-off in tobacco seedlings in Cuba. Fitosanidad 13:221

Godoy-Lutz G, Steadman JR, Higgins B, Powers K (2003) Genetic variation among isolates of the web blight pathogen of common bean based on PCR–RFLP of the ITS-rDNA region. Plant Dis 87:766–771

Godoy-Lutz G, Kuninaga S, Steadman JR, Powers K (2008) Phylogenetic analysis of *Rhizoctonia solani* subgroups associated with web blight symptoms on common bean based on ITS-5.8S rDNA. J Gen Plant Pathol 74:32–40

González García M (2008) Aspectos de sistemática y biología del complejo *Rhizoctonia*. Fitosanidad 12:147–159

González M, Pujol M, Metraux J, González-García V, Bolton M, Borrás-Hidalgo O (2011) Tobacco leaf spot and root rot caused by *Rhizoctonia solani* Künh. Mol Plant Pathol 12(3):209–216

Gurkanli CT, Ozkoc I (2011) First report of B.N. *Rhizoctonia* from Tobacco (*Nicotiana tabacum* L.) in Samsum, Turkey. Pak J Bot 43:51–57

Gurkanli CT, Ozkoc I, Gunduz I (2009) Molecular and conventional identification and pathogenicity of *Rhizoctonia solani* isolates from tobacco (*Nicotiana tabacum* L.) in Samsun, Turkey. J Phytopathol 157:686–696

Gutierrez WA, Shew HD, Melton TA (1997) Sources of inoculum and management for *Rhizoctonia solani* damping-off on tobacco transplants under greenhouse conditions. Plant Dis 81:604–606

Hall T (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symposium Series 41:95–98. Available at: http://www.mbio.ncsu.edu/bioedit/bioedit.html. Accessed 6 Oct 2014

Johnk JS, Jones RK (1993) Differentiation of population of AG-2-2 of *Rhizoctonia solani* by analysis of fatty acids. Phytopathology 83: 278–283

Jones RK, Belmar SB (1989) Characterization and pathogenicity of *Rhizoctonia* spp. isolated from rice, soybean, and other crops grown in rotation with rice in Texas. Plant Dis 73:1004–1010

Laroche JP, Jabaji-Hare S, Charest PM (1992) Differentiation of two anastomosis groups of *Rhizoctonia solani* by isozyme analysis. Phytopathology 82:1387–1393

Li H, Wu B, Yan S (1998) Aetiology of *Rhizoctonia* in sheath blight of maize in Sichuan. Plant Pathol 47:16–21

Liu ZL, Sinclair JB (1993) Differentiation of intraspecific groups within anastomosis group I of *Rhizoctonia solani* using ribosomal DNA internal transcribed spacer and isozyme analysis. Can J Plant Pathol 12:376–382

Liu ZL, Domier LL, Sinclair JB (1993) ISG-specific ribosomal DNA polymorphism of the *Rhizoctonia solani* species complex. Mycologia 85:795–800

Lucas G (1975) Diseases of Tobacco. 3rd ed. Biological Consulting Associates, Box 5726. Raleigh, North Carolina. Parker and Sons Printers

Masuka AJ (1998) Binucleate *Rhizoctonia* on tobacco in Zimbawe. Plant Dis 82:263

Mercado Cárdenas G, Galván M, Barrera V, Carmona M (2012) First report of target spot of tobacco caused by *Rhizoctonia solani* (AG-2.1). Plant Dis 96:456

Nicoletti R, Lahoz E (1995) Recovery of *Rhizoctonia solani* AG-5 from tobacco in Italy. Plant Dis 79:540

Nicoletti R, Lahoz E, Kanematsu S, Naito S, Contillo R (1999) Characterization of *Rhizoctonia solani* isolates from tobacco fields related to anastomosis groups 2-1 and BI (AG 2-1 and AG BI). J Phytopathol 147:71–77

Page RD (1996) TREEVIEW: an application to display phylogenetic trees on personal computers. Comput Appl Biosci 12: 357–358

Pascual CB, Toda T, Raymondo AD, Hyakumachi M (2000) Characterization by conventional techniques and PCR of *Rhizoctonia solani* isolates causing banded leaf sheath blight in maize. Plant Pathol 49:108–118

Schoch CL, Seifert KA, Huhndorf S, Robert V, Spouge JL, Levesque CA, Chen W, Fungal Barcoding Consortium (2012) Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for fungi. Proc Nat Acad Sci 109:6241–6246

Sharon M, Kuninaga S, Hyakumachi M, Sneh B (2006) The advancing identification and classification of *Rhizoctonia* spp. using molecular and biotechnological methods compared with the classical anastomosis grouping. Mycoscience 47:299–316

Sharon M, Kuninaga S, Hyakumachi M, Naito S, Sneh B (2008) Classification of *Rhizoctonia* spp. using rDNA-ITS sequence analysis supports the genetic basis of the classical anastomosis grouping. Mycoscience 49:93–114

Sherwood RT (1969) Morphology and physiology in four anastomosis groups of *Thanatephorus cucumeris*. Phytopathology 59:1924–1929

Shew HD, Lucas GB (1991) Compendium of tobacco diseases. The American Phytopathological Society Press, St. Paul

Shew HD, Melton TA (1995) Target spot of tobacco. Plant Dis 79:6–11

Sneh B, Burpee L, Ogoshi A (1991) Identification of *Rhizoctonia* species. The American Phytopathological Society, APS Press, EE.UU

Stenglein SA, Balatti PA (2006) Genetic diversity of *Phaeoisariopsis griseola* in Argentina as revealed by virulence and molecular markers. Physiol Mol Plant Pathol 68:158–167

Swofford DL (2002) PAUP*. Phylogenetic analysis using parsimony (*and other methods), Version 4. Sinauer Associates, Sunderland, Massachusetts, USA

Tarantino P, Caiazzo R, Carella A, Lahoz E (2007) Control of *Rhizoctonia solani* in a tobacco-float system using low rates of iprodione and iprodione-resistant strains of *Gliocladium roseum*. Crop Prot 26: 1298–1302

Thompson JD, Higgins DG, Gibson TJ (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res 22:4673–4680

Thorn RG, Reddy CA, Harris D, Paul EA (1996) Isolation of saprophytic Basidiomycetes from soil. Appl Environ Microbiol 62:4288–4292

Tu C, Hsieh T, Chang Y (1996) Vegetable diseases incited by *Rhizoctonia* sp. In: Sneh B, Jabaji-Hare S, Neate S, Dijst G (eds) *Rhizoctonia* Species: taxonomy, molecular biology, ecology, pathology and disease control. Kluwer Academic Publishers, Dordrecht, pp 369–377

White TJ, Bruns T, Lee S, Taylor JW (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In:

Innis MA, Gelfand DH, Sninsky JJ, White TJ (eds) PCR protocols: a guide to methods and applications. Academic Press Inc, New York, pp 315–322

Zhao Y, Wu Y, Fu Y, An M, Chen J, Zhao X (2013) Characterization of *Rhizoctonia solani* AG-3 isolates causing Target Spot of Flue-cured Tobacco in China. Adv Mater Res 17:4321–4325