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

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

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ABSTRACT

In Argentina, over 60% of the tobacco crop is grown in the northwestern part of the country. In this region, *Rhizoctonia solani* can cause serious disease problems that result in losses in yield and quality. In the present study ~~W~~we collected 35 isolates of *Rhizoctonia* from 32 tobacco fields in ~~Northwestern~~ northwestern Argentina and characterized the isolates by means of 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. ~~Greater~~ ~~m~~Morphological variability was greater ~~observed~~ within isolates of AG 2-1 and AG 4 HG-III than ~~in isolates~~within those of AG 4 HG-I. The aggressiveness of isolates on tobacco seedlings was determined under greenhouse conditions. Isolates of AG 2-1 were the most aggressive on leaves (causing target spot), whereas~~ile~~ isolates of AG 4 HG-I were the most aggressive on stems and roots (~~,~~causing damping-off). ~~This is the first in depth study of *Rhizoctonia* on Virginia tobacco cultivars in the major production areas of Argentina.~~

Key words: *Nicotiana tabacum*, ITS, anastomosis group (AG), vegetative compatibility.

INTRODUCTION

Tobacco ~~Commercial production of tobacco~~ (*Nicotiana tabacum* L.) is commercially produced~~occurs~~ in more than 128 countries worldwide. Argentina is ~~in the top 10 unmanufactured tobacco producing country~~among the ten main producers of unmanufactured tobacco~~ies of unmanufactured tobacco~~, with over 60% of the ~~Argentinean~~ tobacco grown in its~~the~~ northwestern region ~~of the country~~ (FAO, 2012). Production is mainly in the provinces of Salta and Jujuy, with a mean yield of 2000 kg ha^{-1}. Principal 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 ~~Different~~ *Rhizoctonia* species ~~were~~ reported as pathogenic to tobacco, we can mention. ~~M~~ multinucleate species such as ~~include~~ the teleomorphs *Thanatephorus cucumeris* (*Rhizoctonia solani*) and *Waitea circinata* var. *zeae* (*Rhizoctonia zeae*), and binucleate species such as~~include~~ *Ceratobasidium* spp. (Lucas, 1975; Nicoletti and Lahoz, 1995; Shew and Melton, 1995; Garcia et al., 2009; Gurkanli and Ozkoc, 2011). These pathogens have been reported as associated to tobacco diseases like target spot, stem rot and damping-off in transplants and target spot and sore shin in field plants causing crop losses of up to 15% (Lucas, 1975; Shew and Lucas, 1991; Csinos and Stephenson, 1999; Tarantino, 2007; Gonzalez et al., 2011). Target spot disease requires high~~Since target spot demands high r~~ relative humidity and rainfall, which, when ~~is less~~ frequent, can result in very severe epidemics and losses. However, these conditions are not frequent in tobacco growing areas worldwide (Sherwood, 1969; Shew and Melton, 1995; Gurkanli et al., 2009)~~, but where present, can result in very severe epidemics and losses (Shew and Melton, 1995)~~.

~~Classification of isolates w~~Within *R. solani*, c-lassification of isolates is based on the mycelial compatibility or incompatibility between isolates, which is used to place isolates into

anastomosis groups (AGs) (Sneh et al., 1991; Carling, 1996). Currently, a total of 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 ~~in~~ 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 ~~were~~ have also been reported as causes 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 demands the availability of the tester isolates~~experience and is time consuming~~ (Sharon et al., 2006; Garcia et al., 2009). ~~Moreover, this is not always a precise method, because certain isolates do not anastomose with representatives of any known AG and some isolates have lost their capability to self-anastomose (Hyakumachi and Ui, 1987; Sharon et al., 2006).~~In recent years, the classical AG-grouping has been confirmed by the use of DNA--based techniques.

~~Other techniques like PCR-RFLP and rDNA sequence analysis have been developed for *Rhizoctonia* characterization (Schneider et al., 1997; Guillemaut et al., 2003; Hyakumachi et al., 2005).~~ 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., simplifying the classification of this species complex (Sharon et al., 2006, 2008). Moreover, ITS sequences have been proposed as a ~~universal~~ 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 ~~were~~ have been reported, and, to our knowledge, ~~,~~there are no studies about the identification of the *Rhizoctonia* spp. causing disease in tobacco in

Argentina. Knowledge of the diversity of ~~the~~ this pathogen is important to understand host pathogen interactions and disease epidemiology. Thus, ~~There are no studies about the identification of the *Rhizoctonia* spp. causing disease in tobacco in Argentina. i~~In order to develop a successfully integrated control program to minimize yield losses due to *Rhizoctonia* diseases, the aims of this work were to identify the *Rhizoctonia* spp. associated with tobacco diseases in northwestern Argentina and to assess their aggressiveness towards tobacco under greenhouse conditions.

MATERIALS AND METHODS

Survey and fungal isolation

A survey was conducted in ~~Northwestern~~ northwestern Argentina tobacco fields during the 2008-2011 growing seasons to identify the *Rhizoctonia* spp. associated with tobacco. Plants with damping-off, sore shin or target spot symptoms were recovered from 32 different fields~~, following a "W" sampling pattern through each field~~ (Table 1). A total of 39~~25~~ isolates were obtained: 25 from symptomatic tobacco plants, 10 ~~isolates were obtained~~ from soil (described below), and four ~~isolates were obtained~~ from rotation crops with symptoms of disease caused by *Rhizoctonia* (Table 1). Isolation from plant tissues was made on potato dextrose agar medium (PDA; Britania S.A., Argentina) acidified to pH 5 with 10% lactic acid and incubated at 25 ± 2°C in darkness for 2 to 3 days. Isolation~~s~~ from soil ~~were obtained~~was made as described by Alfenas et al. (2007). Briefly, 100 g of soil ~~were~~ 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 25ºC for 24-48 hours, 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 to 24 hours. 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 days (Table 2). Isolates were placed into 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 "Laboratorio de Sanidad Vegetal" INTA-EEA-Salta Microbial Collection, Argentina.

~~The effect of temperature on radial growth was determined at four temperatures (10, 20, 30 and 40°C). Mycelial plugs (0.2 cm diameter) were transferred from the edge of 5-day-old actively growing colonies on PDA to new dishes of PDA. Dishes were incubated in the dark and colony radius was measured daily in four directions. Each isolate was replicated five times at each temperature and the entire test was repeated twice. Descriptive analysis and graphical representations were applied for data analysis.~~

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., 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 in a thermocycler (Eppendorf Master Cycler , Hamburg, Germany) programmed with an initial denaturing step at 94°C for 1 min, followed by 30 cycles at 94°C for 15 sec, annealing at 58°C for 15 sec and 72°C for 15 sec, 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 a purification kit (QIAquick PCR Purification Kit, Qiagen Inc., Stanford, CA, USA). The purified products were subjected to sequencing in both directions using primers ITS1 and ITS4 in an ABI 3130XL DNA sequencer (Applied Biosystems, CA, USA).

Pathogenicity determination

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 ~~moistered~~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 one 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 m~~M~~orphological characteristics of the pathogen reisolated 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 ~~anastomosis groups~~AGs. Isolates from other hosts (bean, chickpea, sorghum and peanut) were included in the analysis. ~~*Athelia rolfsii* was used as an outgroup.~~ 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 ~~neighbour~~ 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. ~~Only values above 50 are reported.~~

RESULTS

Morphological characterization

Isolations from diseased tobacco, soil, and rotation crops resulted in recovery of 39 isolates of *Rhizoctonia*; 36 isolates were multinucleate and three were binucleate (Table 1). The number of nuclei per cell ~~varied from two to eight for~~ in the multinucleate isolates varied

from two to eight. All cells of the binucleate *Rhizoctonia* isolates contained only two 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 hours. After two 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. ~~Morphological groups~~MG 1 and 2 included all the *R. solani* isolates analyzed. ~~Isolates within morphological groups differed in their growth rate on PDA (Figure 1). Isolates in MG-3 had the highest rate of growth, while isolates in MG-4 had the lowest. Only isolates in MG-1 and MG-2 grew at all temperatures, with optimum temperatures below 30°C and 20°C, respectively (Figure 1).~~

ITS-rDNA phylogeny

Genomic DNA of the 39 *Rhizoctonia* spp. isolates ~~were~~ 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 ~~were~~ identified as *R. solani*, while the rest of the isolates corresponded to four *Rhizoctonia* spp. (Figure ~~2~~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~~ this isolate was not virulent on the tobacco variety tested ~~for pathogenicity determination~~ (Table 1). ~~AGs of the~~The *R. solani* isolates included 13 isolates of AG 2-1, 14 of AG 4 HG-I, four of AG 4 HG-III, and one 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, ~~and~~ whereas 22 isolates caused damping-off symptoms (Table 1). Seven of the isolates caused only target spot, and seven other isolates caused only damping-off. All uninoculated plants remained healthy. ~~Tobacco plants developed~~ T~~t~~ypical symptoms of target spot were developed within two days after inoculation whereas~~and~~ typical symptoms of damping-off or stem rot were developed within five days. Severity ratings ranged from 0 to 4 for both diseases across all isolates~~, with 11~~. Eleven isolates were not pathogenic under the conditions of the test.~~ Seven of the isolates only caused only target spot, and seven other isolates only caused only damping-off. All uninoculated plants remained healthy.~~ 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 (Figure 2~~3~~). For example, isolates of AG 4 were significantly less aggressive than those of AG 2-1 for target spot ($p<0.0001$) (Figure 2~~3~~), whereas~~while~~ those of AG 4 were the most aggressive isolates on stems ($p=0.05$).

DISCUSSION

In the present study~~I~~ isolates of *Rhizoctonia* were recovered from tobacco plants and fields in ~~Northwestern~~ 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. ~~Surveys on *Rhizoctonia*-caused diseases of tobacco have been done in other areas like South America, the USA and Canada (Echavez-~~

Badel et al., 2000; Ceresini et al., 2002; Coyne et al., 2003; LaMondia, 2012). However, this is the first in depth study of the species of *Rhizoctonia* present in Virginia tobacco major production areas of Argentina.

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 tobacco seedlings and fields in Salta and Jujuy provinces, as was 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 Results from our study are in some agreement with these previous reports. Tthe *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,While AG 4 HG-I and AG 4 HG-III isolates were associated with sore shin and damping-off symptoms, respectively. Surprisingly, AG 3 was not detected in the isolates analyzed in spite of beenalthough AG3 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 one isolate of AG 8 from a diseased tobacco plant, but this isolate was nonpathogenic in our inoculation trials.

The pPredominance 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 2-1 and~~ 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 one 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 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 ~~of~~ the *R. solani* isolates were included in only two of our four 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. ~~Higher m~~Morphological variability was higher ~~observed~~ within AG 2-1 and AG 4 HG-III than within isolates of AG 4 HG-I. ~~The slower mycelia growth rate of AG 2-1 isolates compared with that of AG4 (Figure 1), agrees with previous reports (O'Sullivan and Kavanagh, 1991; Schneider et al., 1997; Bacharis et al., 2010).~~

A significant relationship ~~between AG and type of disease symptoms~~ 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 ~~AG 2-1 and AG 4 HG-I causing~~ 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 ~~was~~ 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 the~~i~~se 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 ~~The association of~~ *R. solani* AG 2-1 is associated ~~to~~with target spot o~~i~~n tobacco ~~recently has been report~~ seedlings produced under float systems, but made no field observations (Mercado Cárdenas et al., 2012). ~~Target spot symptoms were only observed in seedlings produced in tobacco float systems and were not observed in the field.~~

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~~Since~~ AGs vary in their sensitivity to fungicides~~, correct identification of pathogens causing disease is important~~ (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., 2010; 2011; Li et al., 1998; Tu et al., 1996). In ~~our~~ the present study, only four 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.

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Table 1. Anastomosis group (AG), isolate code, source, origin, ~~collecting year~~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" ~~seadlings~~seedlings.

Table 2. Morphological groups of *Rhizoctonia* spp. isolates based on mycelia 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./ ~~Rs72~~AG-F** (, Rs71) ***Ceratobasidium* sp./AG-P** (, Rs71b, Rs56), ~~Rs59, Rs59b, Rs59c, Rs58, Rs56, Rs36b, Rs36, Rs25, Rs25b~~	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), ~~Rs73, Rs70, Rs69, Rs68, Rs67, Rs66, Rs63, Rs61, Rs60, Rs49, Rs47,~~	Light brown	Poor	brown	Immersed and aerial

	~~Rs47b, Rs46, Rs46b, Rs44, Rs12~~				
MG-3	***W. circinata* var. *zeae*** (Rs22a, Rs22b)	Light orange	Abundant	Orange	Immersed and aerial
MG-4	~~Rs53b~~***W. circinata* var. *zeae*~~,~~** (-Rs35) ***Rhizoctonia* sp.** (~~,~~-Rs65)	Light brown	Poor	-	-

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

~~**Figure 1.** Radial growth at different temperatures for each morphological group (MG) of *Rhizoctonia* spp. isolates.~~

Figure ~~2~~1. Neighbor-joining tree ~~composed~~ based on~~of~~ rDNA-ITS sequences of *Rhizoctonia* spp. isolates and corresponding sequences from ~~the~~ GenBank. *Athelia rolfsii* was used as an outgroup. Only bootstrap values above 50 are reported. ~~,~~ ~~clustered according to multiple alignment of rDNA-ITS sequence analysis.~~

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

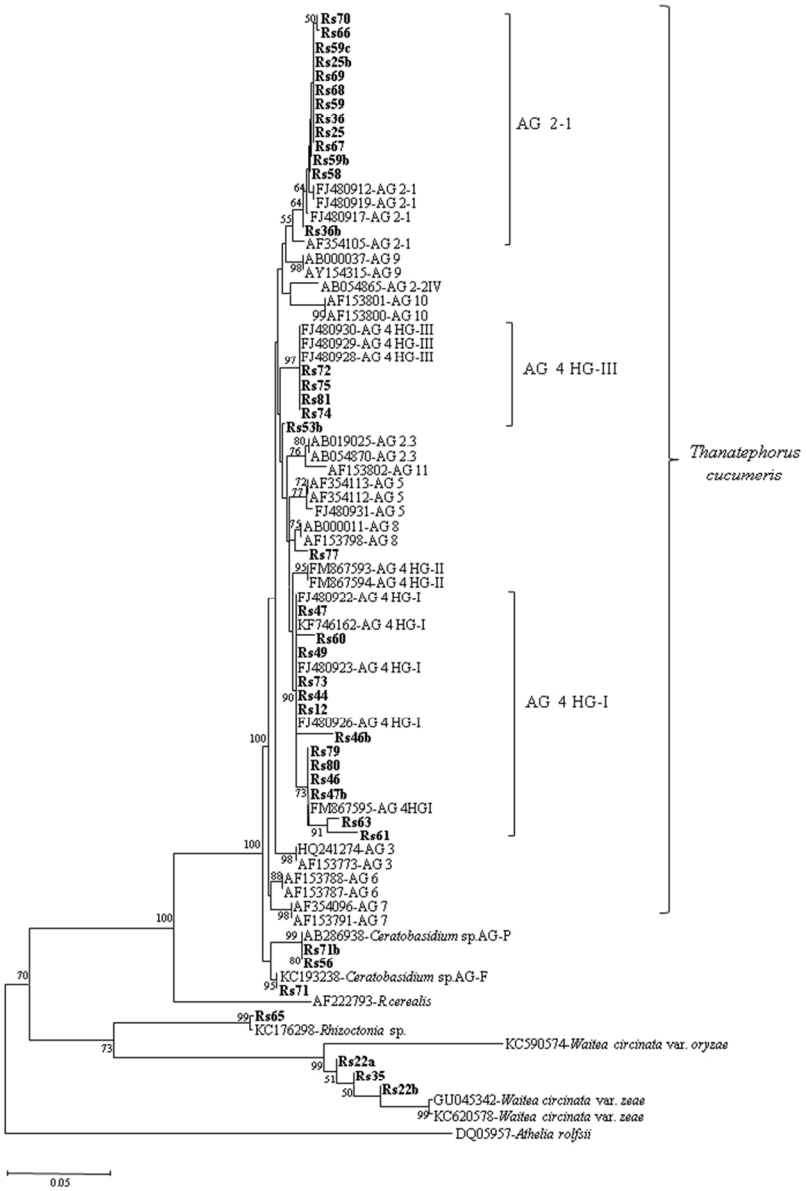

Figure 1. Neighbor-joining tree based on rDNA-ITS sequences of *Rhizoctonia* spp. isolates and corresponding sequences from GenBank. *Athelia rolfsii* was used as an outgroup. Only bootstrap values above 50 are reported.
190x275mm (96 x 96 DPI)

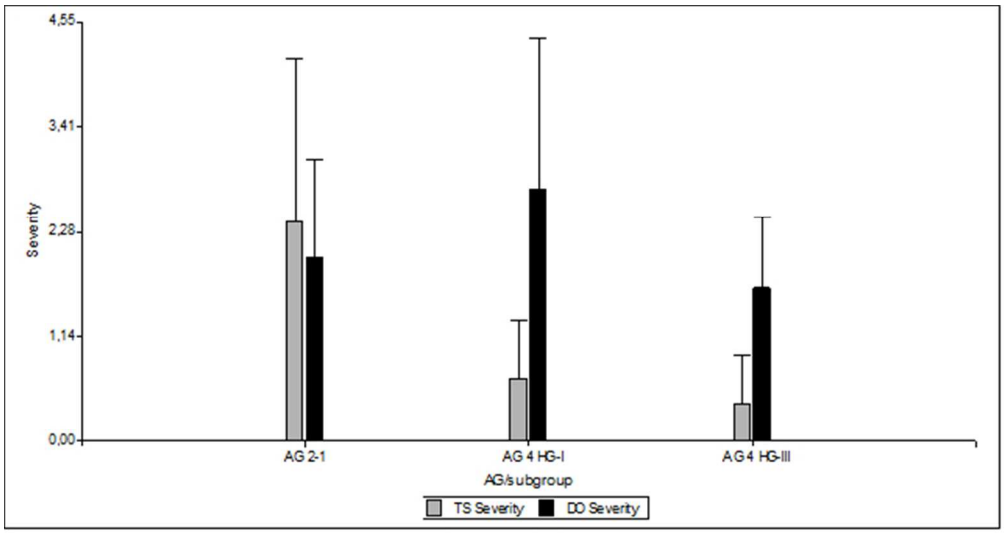

Figure 2. Disease severity index (DSI) of target spot and damping-off of three *Rhizoctonia solani* AGs (AG 2-1, AG 4 HG-I and AG 4 HG-III).
169x89mm (96 x 96 DPI)