Rhizoctonia solani AG-3PT is the major pathogen associated with potato stem canker and black scurf in Colombia

Rhizoctonia solani GA-3PT es el principal patógeno asociado con el chancro del tallo y la sarna negra de la papa en Colombia

Rosa Lilia Ferrucho[1], Johan Manuel Cifuentes[1], Paulo Ceresini[2], and Celsa García-Domínguez[1]

ABSTRACT

Stem canker and black scurf diseases of potatoes are caused by the basidiomycetous fungus *Thanatephorus cucumeris* (anamorphic species complex *Rhizoctonia solani*). These diseases have worldwide distribution wherever potato is grown but their etiology varies depending on the predominance of distinct *R. solani* anastomosis groups (AGs) in a particular area. Within the species complex, several AGs have been associated with stem canker or black scurf diseases, including AG-1, AG-2-1, AG-2-2, AG-3, AG-4, AG-5 and AG-9. This article reports on the most comprehensive population-based study, providing evidence on the distribution of *R. solani* AGs in Colombian potato fields. A total of 433 isolates were sampled from the main potato cropping areas in Colombia from 2005 to 2009. Isolates were assigned to AGs by conventional PCR assays using specific primers for AG-3, sequencing of the ITS-rDNA and hyphal interactions. Most of the isolates evaluated were assigned to AG-3PT (88.45%), and a few to AG-2-1 (2.54%). The remaining isolates were binucleate *Rhizoctonia* (AG-A, E, and I). Pathogenicity tests on the stems and roots of different plant species, including the potato, showed that AG-3PT affects the stems of solanaceous plants. In other plant species, damage was severe in the roots, but not the stems. AG-2-1 caused stem canker of *Solanum tuberosum* cv. Capiro and in *R. raphanistrum* and *B. campestris* subsp. *Rapa* plantlets and root rot in other plants. The results of our study indicated that *R. solani* AG-3PT was the principal pathogen associated with potato stem canker and black scurf diseases of potatoes in Colombia.

Key words: black scurf diagnosis, molecular techniques, soilborne fungi.

RESUMEN

El chancro del tallo y la sarna negra de la papa son ocasionados por el hongo basidiomicete *Thanatephorus cucumeris* (especie anamórfica compleja *Rhizoctonia solani*). Estas dos enfermedades tienen una distribución global en los cultivos de papa, pero su etiología local es variable, dependiendo de la predominancia de diferentes grupos de anastomosis (GAs) en una localidad dada. Al interior de esta especie compleja se han encontrado varios GAs asociados con el chancro del tallo y la sarna negra, como GA-1, GA-2-1, GA-2-2, GA-3, GA-4, GA-5 y GA-9. Este artículo presenta el estudio más exhaustivo a nivel poblacional realizado en Colombia sobre la distribución de los GAs de *R. solani* en lotes comerciales de papa. Se examinaron 433 aislamientos colectados entre 2005 y 2009 en las regiones paperas más importantes del país. Los aislamientos fueron asignados a los GAs por PCR usando cebadores específicos para el GA-3 y secuenciamiento del ITS-ADNr e interacciones hifales. El GA-3PT cubrió la mayoría de los aislamientos (88.45%), el GA-2-1 tuvo el 2.54% y los aislamientos restantes correspondieron a cepas binucleadas de *R. solani* (GA-A, -E, y -I). Las pruebas de patogenicidad sobre diferentes especies de plantas, incluida la papa, evidenciaron que el GA-3PT afecta los tallos de las plantas de la familia Solanácea; en otros hospedantes evaluados el daño fue severo en las raíces pero no en los tallos; el GA-2-1 ocasionó chancros en tallos de plántulas de *Solanum tuberosum* cv. Capiro y *R. raphanistrum* and *B. campestris* subsp. *Rapa*, y pudrición de raíces en otras plantas. Los resultados de nuestro estudio indican que el GA-3PT de *R. solani* es el principal patógeno asociado con las enfermedades del chancro del tallo y la sarna negra de la papa en Colombia.

Palabras clave: diagnóstico de la sarna negra, técnicas moleculares, hongos del suelo.

Introduction

Rhizoctonia solani Kühn [teleomorph *Thanatephorus cucumeris* (Frank) Donk] is a destructive soilborne pathogen that causes diseases in many plant species world-wide (Ogoshi, 1996). Symptoms associated with *R. solani* are diverse, including damping-off, root rot, stem canker, crown rot and blights (Anderson and Stretton, 1982; Ogoshi, 1996). *Rhizoctonia* species have been traditionally identified based on the cell nuclear condition (multinucleate, binucleate and uninucleated strains). *R. solani* is a

Received for publication: 23 July, 2011. Accepted for publication: 29 June, 2012.

[1] Laboratorio de Biotecnologia Antonio Angarita Zerda (LBAAZ), Faculty of Agronomy, Universidad Nacional de Colombia. Bogota (Colombia). cgarciad@unal.edu.co

[2] Departamento de Fitossanidade, Engenharia Rural e Solos, Universidade Estadual Paulista (UNESP). Sao Paulo (Brasil).

species complex consisting of several distinct Anastomosis Groups (AGs) that vary in hyphal anastomosis ability, host range, symptoms in different hosts, geographical distribution, biochemical patterns, fatty acids composition and nuclear DNA sequence (Anderson and Stretton, 1982; Ogoshi, 1987; Carling, 1996; Cubeta and Vilgalys, 1997). AGs have been proposed to represent an independent evolutionary unit or phylospecies within the *R. solani* species complex (González *et al.*, 2006).

Traditionally, AGs have been identified based on the ability of fungal hyphae to anastomose with tester isolates of designated AGs (Carling, 1996; Sneh *et al.*, 1998; McCabe *et al.*, 1999). The methodology for AG-grouping based on microscopical somatic interactions is time consuming and its implementation requires experience (Lees *et al.*, 2002). Alternative methodologies have been developed to improve AG identification with large-scale population-based sampling. Currently, sequence analyses of ribosomal DNA (rDNA) regions are used as a simple and reliable methodology for the accurate molecular systematics of *Rhizoctonia* species and their anastomosis groups (Kuninaga *et al.*, 1997; Sharon *et al.*, 2006; Sharon *et al.*, 2008).

Currently, 14 AGs have been described and named AG-1 through AG-13 and AG-BI (Carling *et al.*, 2002; Truter and Wehner, 2004; Sharon *et al.*, 2008). The first AGs described (AG-1, -2, -3 and -4) were associated with the most destructive Rhizoctonia diseases around the world, whereas those described later are regarded as less destructive pathogens and have more restricted geographical distribution (Carling *et al.*, 2002). The majority of potato (*Solanum tuberosum* L.)-infecting *R. solani* isolates described worldwide belong to AG-3 subgroup PT (AG-3PT), although AG-1, AG-2-1, AG-2-2, AG-4, AG-5 AG-7 and AG-9 have also been associated with disease symptoms on potato stems, stolons, roots and tubers in several other places around the world (Anguiz and Martin, 1989; Bains and Bisht, 1995; Balali *et al.*, 1995; Virgen-Calleros *et al.*, 2000; Campion *et al.*, 2003; Truter and Wehner, 2004; Rosa *et al.*, 2005; Woodhall *et al.*, 2007; Tsror, 2010; Cedeño *et al.*, 2001). The main diseases caused by AG-3PT of potatoes are black scurf on tubers and stem canker. These two diseases are economically important, occurring in potato production areas throughout the world (Banville *et al.*, 1996; Jeger *et al.*, 1996; Banville and Carling, 2001).

R. solani is mainly disseminated via contaminated tubers. Once the fungus is established in a particular area, inoculum builds up with consecutive seasons of potato monoculture (Tsror and Peretz-Alon, 2005; Tsror, 2010). *R. solani* can cause both quantitative and qualitative damage to potato crops. Quantitative losses are due to stem, stolon and root infections, which affect tuber size and number. Qualitative losses are mainly due to deformed and cracked tubers and to the development of sclerotia on the tuber surface (Banville, 1989; Carling *et al.*, 1989; Hartill, 1989; Scholte, 1989; Platt *et al.*, 1993; Jeger *et al.*, 1996; Tsror, 2010). Initial infection of sprouts prior to emergence causes lesions and may be lethal to sprouts or sprout tips. However, the emergence of new compensatory sprouts may result in no significant crop damage (Lehtonen *et al.*, 2008).

In Colombia, the potato is grown in the Andean highlands (2,500-3,200 m a.s.l.). The departments with the largest production areas are Cundinamarca, Boyaca, Nariño and Antioquia. They contribute 70% of the national potato production (Espinal *et al.*, 2006). Seed production is mainly domestic. Although some potato growers use certified tuber seed, it is impossible to guarantee *R. solani*-free tubers mainly because current Colombian regulations for seed potato production allow up to 10% sclerotia-infected tubers (ICA, 2003). In consequence, the pathogen is easily dispersed between regions on infested seed tubers. Research on Rhizoctonia diseases associated with potatoes in Colombia has focused on biological control strategies (Bautista *et al.*, 2007; Beltrán *et al.*, 2007). Knowledge about the disease biology and the relative importance of the various AGs on the etiology of Rhizoctonia diseases in potatoes is still scarce. Since farmers, in general, are not aware of the underground symptoms on potato stems, there are no estimates of the importance of the Rhizoctonia diseases on tuber quality and yield. The main goal of this research was to determine the etiology of Rhizoctonia diseases of potatoes based on a large-scale population sampling in Colombia. Previous studies have shown that AGs other than AG-3 PT may occur in lower proportion in potato fields around the world. The specific objectives of this study were (i) to identify and characterize the relative importance of the distinct *R. solani* AGs associated with potato stem canker and black scurf diseases in Colombia using both a PCR-based method and the classical somatic compatibility interactions for AG-grouping; and (ii) to determine the pathogenicity of each AG in potato and other plant species.

Materials and methods

Population sampling and establishment of a fungal isolate collection

Stem canker-diseased early-sprouting potato plants were sampled from six to eight transects within fields from six

distinct fields in Cundinamarca, four in Boyaca, two in Nariño, Antioquia and Santander, and one in Cauca and Norte de Santander (Fig. 1, Tab. 1). These fields were located across the Colombian Andes and represent the most important potato cropping areas in Colombia (Espinal *et al.*, 2006).

These samples were brought to the Laboratorio de Biotecnología Antonio Angarita Zerda (LBAAZ) of the Facultad de Agronomía at the Universidad Nacional de Colombia. The plants were washed in tap water and infected stem segments were plated on a modified selective medium for *R. solani* (Ko and Hora, 1979), modified by Castro *et al.* (1988). Samples were incubated at room temperature (20°C) in the dark. Whenever possible, seed tubers were also collected with the purpose of isolating the fungus from sclerotia. After 24 to 48 h, pure cultures of *R. solani* were established by transferring hyphal tips from the growing colonies on the selective medium onto potato dextrose agar medium (PDA, Oxoid).

Anastomosis group identification

Mycelium for genomic DNA extraction from 433 isolates was obtained from 5-d-old cultures on PDA containing a sterile cellophane sheet. After incubation at room temperature, mycelium from each isolate was harvested by scraping the mycelia from the cellophane membrane, followed by freezing and lyophilization. DNA was extracted with a QIAGEN® DNeasy Plant Mini-Kit.

The anastomosis group was determined by selective amplification of the ribosomal DNA (rDNA) region using specific primers for *R. solani* AG-3 (Lees *et al.*, 2002). Isolates from Cundinamarca, Antioquia and Nariño were processed at the Institute of Integrative Biology, ETH Zurich. Polymerase chain reactions (PCR) were carried out in 10 µl volumes containing 10-50 ng of genomic DNA, 10 mM of KCl, 10 mM of $(NH_4)_2SO_4$, 20 mM of Tris-HCl, 2 mM of $MgSO_4$, 0.01% of Triton X-100, pH 8.8 (NEB), 0.2 µM of each primer (Microsynth), 0.1 mM of each dNTP and 0.06 U of *Taq* polymerase (NEB). PCR conditions comprised

TABLE 1. Information of the origin of the infected potato populations analyzed in this study.

Departament	Municipality	Field	Geographical coordinates	Altitude (m a.s.l.)	Potato species and cultivar	Sampling year
Cundinamarca	Subachoque	1	4º 57´ 26" N 74º 11´59" W	2,931	*S. tuberosum* R-12	2006
		2	4º 57´ 11" N 74º 12´ 56" W	3,095	*S. tuberosum* R-12	2006
	Cogua	1	5º 04´ 32" N 73º 58´33" W	2,730	*S. phureja*	2006
		2	5º 04´ 22" N 73º 58´ 45" W	2,722	*S. phureja*	2006
	Sibate	1	4º 25´ 26" N 74º 15´ 08" W	3,250	*S. tuberosum* ICA-Morita	2006
		2	4º 25´ 16" N 74º 14´56" W	3,296	*S. tuberosum* Parda Pastusa	2006
Nariño	Pasto	1	1° 8' 41" N 77° 20' 29" W	3,095	*S. tuberosum* Parda Pastusa	2007
		2	1° 8' 16.73 N 77° 18' 37" W	3,084	*S. tuberosum* Parda Pastusa	2007
Antioquia	La Unión	1	5º 58´ 22" N 75º 22´ 59" W	2,505	*S. tuberosum* Capiro	2008
		2	6º 00´ 26" N 75º 21´ 40" W	2,504	*S. tuberosum* Capiro	2008
Boyacá	Ventaquemada	1	5º 23´ 34" N 73º 27´ 49" W	2,918	*S. tuberosum* Parda Pastusa	2007
		2	5º 25´ 00" N 73º 27´ 25" W	2,907	*S. tuberosum* ICA Unica	2009
	Soracá	1	5º 30´ 09" N 73º 20´ 22" W	2,809	*S. phureja*	2007
		2	5º 30´ 45" N 73º 19´ 33" W	2,849	*S. tuberosum* Capiro	2009
Santander	Carcasí	1	6º 39´418" N 72º 34´610" W	2,950	*S. tuberosum* Parda Pastusa	2008
		2	6º 4´ 470" N 72º 33´181" W	3,296	*S. tuberosum* Parda Pastusa	2008
Norte de Santander	Chitagá	1	7º 03´062" N 72º 40´ 638" W	2,989	*S. tuberosum* Parda Pastusa	2008
Cauca	Silvia	1	2º 31´ 30" N 76º 19´ 09" W	3,238	*S. phureja*	2007

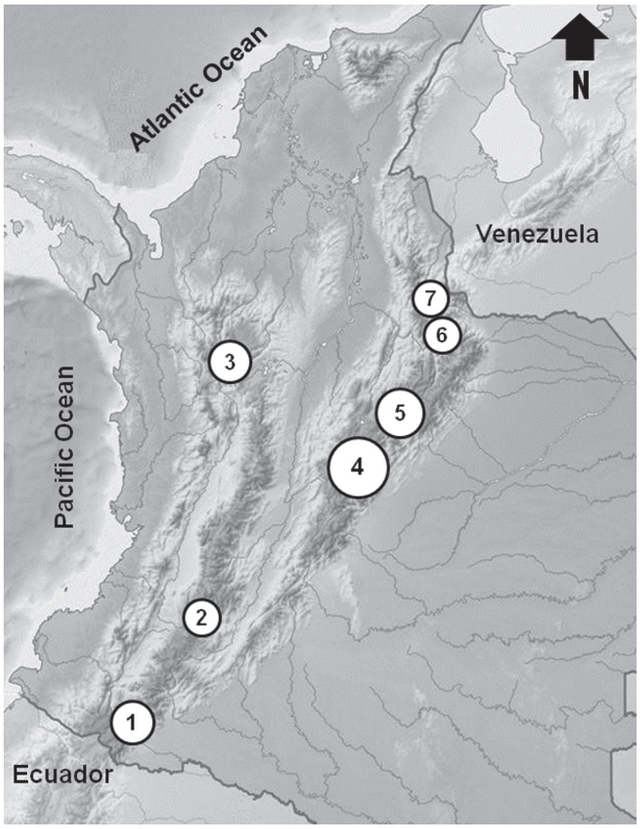

FIGURE 1. Geographical populations of *Rhizoctonia solani* sampled from infested potato fields in Colombia used in this study. The following populations were sampled: 1(Nariño, N=45), 2 (Cauca, N=9), 3 (Antioquia N=63), 4 (Cundinamarca, N=171), 5 (Boyaca, N=110), 6 (Santander, N=21) and 7 (Norte de Santander, N=14). The size of the circle represents the size of the sample.

initial denaturation of 2 min at 96°C, followed by 35 cycles of denaturation for 30 s at 96°C, annealing for 45 s at 57°C and elongation for 45 s at 72°C, with a final extension step of 5 min at 72°C. Isolates collected in Boyaca, Santander, Norte de Santander and Cauca were processed at LBAAZ, following the conditions described previously but using Invitrogen supplies. In both cases, PCR products were visualized with UV on agarose gels.

In order to determine the AG from negative isolates in the amplification with the AG-3 specific primers, the ITS-rDNA region from these isolates and also from the few positive controls were amplified using universal primers ITS5 (5'~GGAAGTAAAAGTCGTAACAAGG~3') and ITS4 (5'~TCCTCCGCTTATTGATATGC~3') (White *et al.*, 1990). Isolates essayed at ETHZ were sequenced using the facilities of the Genetic Diversity Center (GDC) of this institute, while those processed in LBAAZ were sequenced at a commercial laboratory (Macrogen, Seoul). Sequencing reactions were prepared using a BigDye™ v3.0 Cycle Sequencing Kit (Applied Biosystems®, Foster City, CA). Sequencing reactions were run on an ABI PRISM® 3100 genetic analyzer. Sequences were visualized and edited using the program Sequencher 4.0.5 (Gene Codes Corporation, Ann Arbor, MI). The PCR products sent to Macrogen were sequenced in an ABI PRISM® 3730 genetic analyzer and were manually edited using the software ChromasPro 1.5 for Windows (Technelysium Pty. Ltd, Tewantin, Australia).

All the sequences were analyzed with BLAST® (Altschul *et al.*, 1997) against the NCBI sequence database (National Center for Biotechnology Information, GenBank) to detect similar sequences of known AGs. Sequence data of the individuals tested were imported and assembled using BioEdit 5.0.9 (Ibis Biosciences, Carlsbad, CA) (Hall, 1999). Three sequences of *R. solani* AG-3 from GenBank (EF370434, AB19015 and AB19021) were used as references. Nucleotide identity among sequences was determined for each anastomosis group identified.

Microscopic and macroscopic hyphal interactions

Microscopic somatic compatibility reactions were determined with tests using 60 isolates collected in our study and identified as AG-3PT by ITS-rDNA sequencing. Tester isolates belonging to AG-2-1 and AG-3 were used to confirm the AG identity of the isolates essayed. Pairings were made on slides containing a thin layer of water agar (WA Oxoid) and the interaction was evaluated under a light microscope (Carl Zeiss – Axiostar plus), using safranin O staining (Bandoni, 1979). The hyphal anastomosis categories evaluated were those defined previously (Carling, 1996). We observed 15 optical fields and determined the number of fields in each reaction category (C0, C1, C2 and C3), determining the fusion frequency as%FF = (A*100)/B, where A is the sum of fusion points (in C1, C2 and C3 categories) in the 15 microscopic fields and B is the sum of contact points in the 15 microscopic fields (including the four categories) (Sneh *et al.*, 1998).

A set of 21 AG-3PT isolates was tested for macroscopic hyphal interactions to determine the somatic compatibility among isolates collected from different locations. Plates containing PDA were inoculated with disks of colonized PDA removed from the edge of an actively growing colony from the tested isolates and located three cm apart from each other. The 21 isolates were tested in all the possible combinations. Plates were incubated at 15°C and were evaluated after 21 d. The macroscopic somatic reactions were defined as merge and tuft according to defined categories (MacNish *et al.*, 1997).

Pathogenicity tests

Three isolates of each AG were randomly chosen for the pathogenicity test. Prior to inoculation, each isolate was grown for 48 h on PDA. Eleven plants species were tested as potential hosts: (i) seedlings of carrot (*Daucus carota* L.), bean (*Phaseolus vulgaris* L.), corn (*Zea mays* L.), tomato (*Solanum lycopersicum* L.), lulo (*Solanum quitoense* Lam.), pea (*Pisum sativum* L.), ryegrass (*Poa pratensis* L.), and some weeds commonly found in potato fields (*Raphanus raphanistrum* L. and *Brassica campestris* subsp. *rapa* (L.) Hook.); (ii) grass plantlets (*Pennisetum clandestinum* Hoechst Ex Chiov.) rooted in peat. Potato sprouts (*Solanum tuberosum* L. - cv. Capiro) rooting in peat were used as susceptible host plants. Seedlings were transferred to pots containing autoclaved quartz sand 5 d before inoculation. Each plant was inoculated with a 5 mm PDA plug taken from the margins of the fungal colonies (Carling and Leiner, 1986). The mycelial plug was located at one centimeter from the stem and was covered with quartz sand to prevent desiccation. Plants were transferred to a growth chamber and incubated at 20°C/12 h-photoperiod, watered with distilled water every two days and fertilized with nutrient solution (Murashige and Skoog, 1962) once a week. Fifteen days after inoculation, symptoms on the stems and roots were evaluated. In order to characterize differences in aggressiveness among the isolates, two categories of symptoms were considered: (i) on the stems, the symptoms were classified as small superficial lesions (SSL = mild symptoms with lesions size < 5 mm), large superficial lesions (LSL = lesions > 5 mm), or cankers (C); (ii) on the roots, we determined disease incidence, and lesion categories were characterized as on the stems. Symptomatic and non-symptomatic plant tissue was transferred to selective media to check for the presence of the fungus in the tested plants.

Results

Anastomosis group identification by specific PCR and sequencing of ribosomal DNA

Of a total of 433 isolates of *R. solani* collected from symptomatic plants in Cundinamarca, Nariño, Antioquia, Boyaca, Santander and Norte de Santander (Tab. 2), 379 were positive for the AG-3 specific PCR essay (Lees *et al.*, 2002). AG-3 identification based on positive specific PCR amplifications was confirmed by sequencing the ITS-rDNA region from several isolates. In addition, four *R. solani* isolates for which specific PCR amplification failed were classified as AG-3 based on their ITS-rDNA sequence. The sequence of the ITS-rDNA of these four isolates showed a mutation point on nucleotide number nine into the forward primer, which can explain its failure to amplify, these false negatives represent 1% of the evaluated isolates. The ITS1 - ITS2 regions of the rDNA from the *R. solani* sequences in this study showed high sequence similarity (98.3-100%) among themselves and with three AG-3PT sequences from GenBank.

Thus, by the criteria of amplifying with specific PCR primers plus sequencing of the rDNA, 88.45% of the isolates were identified as AG-3. A few isolates were identified as AG-2-1 (2.54%) or binucleate *Rhizoctonia* (AG-A, AG-E and AG-I) (6.24%). Around 3% of the isolates could not be assigned to any anastomosis group, due to poor sequence quality (Tab. 1).

Of the 433 isolates analyzed, 374 were isolated from stem cankers and 59 from sclerotia on mother tubers. Fifty-five tuber-borne sclerotial isolates were identified as AG-3, two as AG-2-1 and two as AG-A and AG-E each.

Hyphal interactions

Microscopic somatic interactions between the AG-3PT tester and a sample of 60 isolates randomly taken from the collection of *R. solani* isolates resulted in positive anastomosis reactions (frequency of hyphal fusion higher than 50%), confirming the classification of isolates based on their ITS-rDNA sequences. Hyphal fusion frequency between isolates belonging to AG-3 varied from 40 to 95%. A high proportion of isolates had frequencies between 45 and 70%, suggesting heterogeneity among individuals. We were not able to distinguish between hyphal perfect fusion (C3) and killing reactions (C2) with microscopic level observations. In the macroscopic somatic essay, the formation of tufts was common (91.4% of isolates pairs evaluated). This was evidence of somatic incompatibility among isolates and only 8.6% of the pairs tested presented merge reactions (perfect fusion). Macroscopic interactions did not reproduce the observed categories of microscopic interactions. In fact, there was a continuum of somatic compatibility to incompatibility (Fig. 2).

Pathogenicity tests

Symptoms observed on the different hosts correspond to typical reported stem canker symptoms (Carling and Leiner, 1986; Carling and Leiner, 1990; Carling *et al.*, 2001), although severity varied between AGs (Tab. 3). Pathogenicity was determined qualitatively and by the estimate of lesion size on the stems and roots. In some cases, isolates showed a distinct host range.

Isolates of *R. solani* AG-3PT significantly affected the stems of solanaceous hosts such as potato, tomato and lulo causing large cankers (> 5 mm). No symptoms were observed on the roots of these plants. Although AG-3PT

TABLE 2. Anastomosis group identification of *Rhizoctonia solani* isolates associated with stem canker and black scurf diseases of potatoes in Colombia.

Department	Municipality	Field	AG-2-1	AG-3	AG-A	AG-E	AG-I	NI	Total
Cundinamarca	Subachoque	1	1	29	1	3	0	0	34
		2	0	20	0	0	1	0	21
	Cogua	1	2	24	3	1	3	0	33
		2	1	36	1	0	0	0	38
	Sibate	1	1	22	0	0	1	0	24
		2	3	15	1	0	1	1	21
Nariño	Pasto	1	0	18	0	2	1	1	22
		2	0	22	0	0	0	0	22
Antioquia	La Unión	1	0	20	3	0	0	0	23
		2	0	35	1	0	0	2	38
Boyaca	Soracá	1	2	24	0	0	0	0	26
		2	1	32	2	0	0	0	35
	Ventaquemada	1	0	20	2	0	0	3	25
		2	0	22	0	0	0	2	24
Santander	Carcasí	1	0	11	0	0	0	1	12
		2	0	10	0	0	0	2	12
Norte de Santander	Chitagá	1	0	14	0	0	0	0	14
Cauca	Silvia	1	0	9	0	0	0	0	9
Total			11	383	14	6	7	12	433
Percentage			2.54	88.45	3.23	1.39	1.62	2.77	

NI= Non-identified

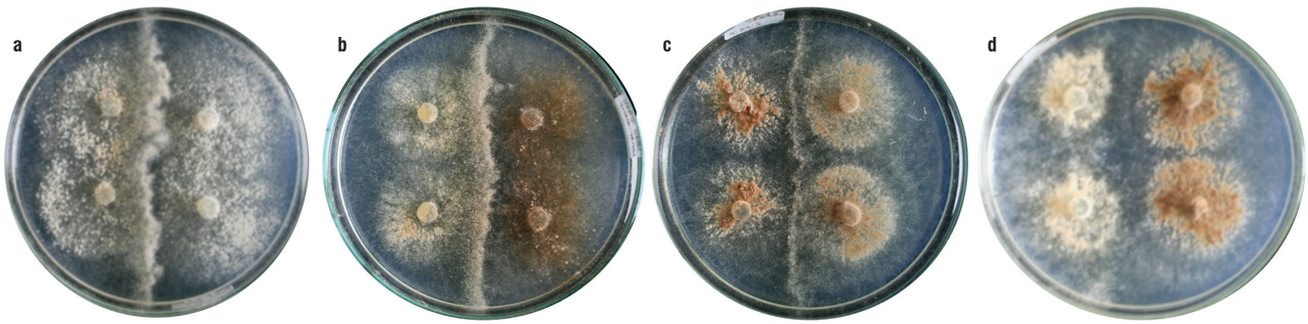

FIGURE 2. Macroscopic anastomosis reaction between *Rhizoctonia solani* AG-3PT isolates. (a) Strong tuft reaction between isolates collected in the same field; (b) strong tuft reaction between isolates collected in different fields; (c) mild tuft reaction between isolates collected in different fields; and (d) merge reaction between isolates collected from different fields. Numbers underneath each figure are codes for isolate pairs. Source photographic: Ferrucho, R.L.

isolates caused very abundant small lesions on the roots of pea, bean, corn and carrot plants, they did not cause lesions on the stems. The other plants inoculated with AG-3PT (*B. campestris* subsp. *rapa*, *R. raphanistrum, P. clandestinum* and *P. pratensis*) did not express symptoms. Besides affecting potatoes, isolates of *R. solani* AG-2-1 attacked the stems of lulo and Brassicaceae hosts (*R. raphanistrum* and *B. campestris* subsp. *rapa*), causing cankers from 0.4 to 10.0 mm, but not on the roots. However, these isolates produced small spots on a large proportion of pea, bean, corn and carrot roots. The two grasses evaluated (*P. clandestinum* and *P. pratensis*) did not show symptoms when inoculated with AG-2-1 or AG-3PT. The fungus was recovered from all the evaluated plants (symptomatic and non symptomatic).

Stem and root lesions were categorized as follows: NI (non-infected); SSL (superficial small lesion) and C (canker). Size of the lesions is the average of 5 plants. The data correspond to the range of symptoms in five inoculated plants for each AG tested. For example, in the same set of plants, we found non-infected plants and plants with cankers (NI/C)

Symptoms caused by binucleate *Rhizoctonia* strains (AG-A, AG-E and AG-I) were characterized by the presence of many small superficial lesions on the roots in all plant species evaluated except for *R. raphanistrum* and *B. campestris* subsp. *rapa*. The stems of the other plants were not affected by any of the binucleate strains tested.

TABLE 3. Host range and pathogenicity of *Rhizoctonia solani* AG-2-1 and AG-3 isolates obtained from commercial potato fields in Colombia.

Host plants	AG-2-1				AG-3			
	Stem lesion	Size (cm)	Root lesion	Diseased roots (%)	Stem lesion	Size (cm)	Root lesion	Diseased roots (%)
Pisum sativum	SSL	< 0.01	SSL	34.5	NI	0	SSL/C	18.6
Phaseolus vulgaris	NI	0	SSL	45.3	NI	0	SSL	31.2
Solanum quitoense	SSL	0.40	NI	0	SI/C	0.70	NI	0
Zea mays	NI	0	SSL	40	SSL	0.40	SSL	60.0
Raphanus raphanistrum	C	0.83	NI	0	NI	0	NI	0
Brassica campestris subsp. *Rapa*	C	0.61	NI	0	NI	0	NI	0
Solanum tuberosum cv. *Capiro*	C	1.03	NI	0	C	1.05	NI	0
Pennisetum clandestinum	NI	0	NI	0	NI	0	NI	0
Poa pratensis	NI	0	NI	0	NI	0	NI	0
Solanum lycopersicum	NI	0	C	0	NI/C	0.62	NI	0
Daucus carota	C	< 0.01	NI/C	7.4	NI	0	NI/C	49.9

Discussion

This is the first large scale population study carried out in Colombia aimed at determining the composition of AGs associated with Rhizoctonia diseases of potatoes and at assessing the effect of the various AGs on symptom expression in different hosts.

Previous reports indicated that *R. solani* AG-3 is the most frequent AG associated with stem canker of potatoes worldwide (Carling and Leiner, 1990; Banville and Carling, 2001; Tsror, 2010). Our results indicated that the Colombian population of *R. solani* was not an exception as the largest proportion of the potato-infecting isolates were identified as AG-3, based on specific PCR essay, sequencing of the ITS-rDNA region and confirmed by anastomosis grouping. The two most efficient and accurate methodologies to assign isolates to AGs were the specific PCR essay and ITS-rDNA sequencing.

Correlation between microscopic killing anastomosis (C2 reaction) and perfect fusion (C3 reaction) with the corresponding macroscopic somatic interactions 'tuft' and 'merge' has been demonstrated for AG-8 (MacNish *et al.*, 1997) and for *R. solani* AG-3PT and TB from potatoes and tobacco (Ceresini *et al.*, 2002). However, the macroscopic somatic interactions were not good predictors for the microscopic anastomosis reactions between isolates of the soybean-infecting pathogen *R. solani* AG-1 IA (Campos and Ceresini, 2006).

Particularly in our study, because we were not able to easily distinguish between perfect fusion and killing anastomosis reaction at microscopic level, we could not establish a correlation between microscopic and macroscopic interactions between isolates of *R. solani* AG-3PT. Consequently, we took the macroscopic interactions as the sole predictor of somatic incompatibility. The macroscopic interactions indicated the predominance of somatic incompatibility among *R. solani* isolates. We also observed that macroscopic interaction phenotypes among isolates varied in intensity. For example, some pairings showed a strong "barrage"-type somatic incompatibility reaction in the line of contact among isolates, which was characterized by a high amount of aerial mycelia (tuft) (Fig. 3 a, b). In contrast, some pairings showed a light but well defined somatic incompatibility reaction. Around 91% of the pairings were somatically incompatible, indicating a high level of genetic diversity among the isolates, at least for the genes related with somatic compatibility. Nine percent of the pairings were somatically compatible, characterized by a "merge"-type interaction. Most of the somatically compatible pairings occurred between isolates from the same county, except for one isolate from Pasto1 which was somatically compatible with five isolates, each one with a different geographical origin.

R. solani AG-3PT was the principal and most aggressive pathogen associated with potatoes in Colombia. The prevalence of AG-3PT of potatoes in Colombia is in accordance with previous worldwide reports (Anguiz and Martin, 1989; Carling *et al.*, 1989; Bains and Bisht, 1995; Balali *et al.*, 1995; Virgen-Calleros *et al.*, 2000; Campion *et al.*, 2003; Truter and Wehner, 2004; Woodhall *et al.*, 2007; Woodhall *et al.*, 2008; Tsror, 2010; Cedeño *et al.*, 2001). *R. solani* AG-2-1 was also detected on stem cankers, but at a much lower frequency and causing only mild symptoms of potatoes. AG-2-1 was detected exclusively in Boyaca and Cundinamarca. The restricted occurrence of AG-2-1 in Boyaca and Cundinamarca may be attributed to the abundance of two Brassicaceae weed species (*R. raphanistrum* and *B. campestris* subsp. *rapa*) (Arrieta, 2000), which are invasive of potato fields and hosts to the pathogen. In fact, AG-2-1 has been reported as a principal *Brassica* pathogen (Carling and Leiner, 1986).

The fact that distinct *R. solani* AGs can be associated with potato stem canker in the same field in Colombia is particularly important for defining disease management strategies effective against all AGs, as their response to a particular strategy can vary (Campion *et al*., 2003). Any attempt for officially labeling fungicides for potato crops should consider the complex etiology of the stem canker disease.

We failed to detect the occurrence of AG-4, -5, -7 and -8 in Colombian potato fields despite the fact that their occurrence has been previously reported for potatoes elsewhere in the world (Anguiz and Martin, 1989; Bains and Bisht, 1995; Balali *et al*., 1995; Virgen-Calleros *et al*., 2000; Cedeño *et al*., 2001; Campion *et al*., 2003; Truter and Wehner, 2004; Rosa *et al.,* 2005; Woodhall *et al*., 2007; Silva-Barreto *et al.,* 2010; Tsror, 2010).

The association of different AGs of *R. solani* with underground diseases in invasive plants has been established in potato fields (Silva-Barreto *et al.,* 2010). Besides environmental factors, the distinctively variable geographical distribution of host plants (such as cultivated, invasive weeds or wild plant species) are probably the most important selection factors determining the occurrence and prevalence of distinct AGs in potato fields. Because crop rotation is one of the main strategies for controlling stem canker and black scurf diseases of potatoes, disease management strategies based on crop rotation must consider the odds of the emergence of a new pathogen of potatoes, especially when the rotation of the crop is susceptible to other *Rhizoctonia*-like pathogens.

Although many *R. solani* AGs are considered host specific, even the host specialized ones have shown some level of damage in hosts distinct from their original host (Anderson and Stretton, 1982; Ogoshi, 1987). Our results indicated that *R. solani* AG-3 is able to infect different plant species. Potatoes, tomatoes and lulo exhibited stem cankers while peas, beans, corn and carrot showed root infection. Despite the lower disease severity levels and organ specificity on other hosts, these plants may contribute to the fungal inoculum build up in the soil. Therefore, the ability of other host plants to harbor AG-3PT or AG-2-1 is another relevant factor underlying the adoption of a crop rotation system. The adoption of a crop rotation system should consider non-host plant species for the pathogens, such as ryegrass, which was not affected by AG-2-1 or AG-3PT, although the fungal inoculum remained viable on plant debris.

Binucleate *Rhizoctonia* species (AG-A, AG-E and AG-I) were also associated with stem canker lesions. These binucleate *Rhizoctonia* appear to have a wide ecological role. Some non-pathogenic *Rhizoctonia* have a role as a biological control agent causing only a mild infection in plants, which triggers defense responses against pathogenic *Rhizoctonia* (Carling and Leiner, 1986; Poromarto *et al*., 1998). Other binucleate *Rhizoctonia* can cause very destructive diseases, such as damping off (Martin, 1988; Escande and Echandi, 1991; Demirci *et al*., 2002). The binucleate *Rhizoctonia* associated with potatoes in Colombia were only mildly pathogenic as symptoms were restricted to the stem surface and no cankers were observed. These strains could be potentially exploited as bio-control agents, although an in-depth study of their ecological role is warranted.

Finally, our research pointed towards the need to evaluate the variation in the reaction of potato germplasm to develop management strategies based on disease resistance.

Conclusions

Specific PCR-based methods or DNA sequencing of the ITS-rDNA were useful for detection and identification of AGs.

R. solani AG-2-1 and AG-3PT were the only two AGs associated with potato stem canker and black scurf diseases in Colombia.

AG-3PT was the principal stem canker and black scurf associated pathogen on potatoes.

AG-2-1 and AG-3PT were able to infect other host plants such as tomatoes, lulo, beans, carrot and peas. AG-2-1, particularly, was pathogenic to Brassicacea plant species.

The adoption of a crop rotation system should consider non-hosts of AG-2-1 and AG-3PT.

Acknowledgments

This research was supported by the Universidad Nacional de Colombia, Ministerio de Agricultura y Desarrollo Rural de Colombia and Asociación Hortifrutícola de Colombia (Asohofrucol), and partially funded by an ETH Zurich (Swiss Federal Institute of Technology, Zurich, Switzerland) grant (TH-16/06) to P. C. Ceresini. Sequence data were collected using the facilities of the Genetic Diversity Center at the ETH Zurich. The primary author wants to thank Dr. Enrique Torres (Emeritus Professor, Universidad Nacional de Colombia) for his review of the manuscript. We also thank Marcello Zala (ETH Zurich) for his

technical assistance. P. C. Ceresini is a Research Fellow (Pq-2 308394/2009-7) from CNPq (Conselho Nacional de Desenvolvimento Cientifico e Tecnologico, Brazil).

Literature cited

Altschul, S.F., T.L. Madden, A.A. Schäffer, J. Zhang, Z. Zhang, W. Miller, and D.J. Lipman. 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucl. Acids Res. 25, 3389-3402.

Anderson, N.A. and H.M. Stretton. 1982. The genetics and pathology of *Rhizoctonia solani*. Annu. Rev. Phytopathol. 20, 329-347.

Anguiz, R. and C. Martin. 1989. Anastomosis groups, pathogenicity and other characteristics of *Rhizoctonia solani* isolated from potato in Peru. Plant Dis. 73, 99-201.

Arrieta, J.M. 2000. Manejo integrado de malezas en el cultivo de la papa. pp. 42-158. In: Herrera, C.A., L.H. Fierro, and J.D. Moreno (eds.). Manejo integrado del cultivo de la papa. Technical Manual. Produmedios, Bogota.

Bains, P.S. and V.S. Bisht. 1995. Anastomosis group identity and virulence of *Rhizoctonia solani* isolates collected from potato plants in Alberta, Canada. Plant Dis. 79, 241-242.

Balali, G.R., S.M. Neate, E.S. Scott, D.L. Whisson, and T.J. Wicks. 1995. Anastomosis group and pathogenicity of isolates of *Rhizoctonia solani* from potato crops in South Australia. Plant Pathol. 44, 1050-1057.

Bandoni, R.J. 1979. Safranin O as a rapid nuclear stain for fungi. Mycologia 71, 873-874.

Banville, G.J. 1989. Yield losses and damage to potato plants caused by *Rhizoctonia solani* Kuhn. Amer. Potato J. 66, 821-834.

Banville, G.J., D.E. Carling, and B.E. Otrysko. 1996. Rhizoctonia disease on potato. pp. 321-330. In: Baruch, S., J.-H. Suha, S. Neate, and G. Dijst (eds.). *Rhizoctonia* species: taxonomy, molecular biology, ecology, pathology and disease control. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Banville, G.B. and D.E. Carling. 2001. Rhizoctonia canker and black scurf. pp. 36-37. In: Stevenson, W.R., R. Loria, G. Franc, and D.P. Weingartner (eds.). Compendium of potato diseases. APS Press, St. Paul, MN.

Bautista, G., H. Mendoza, and D. Uribe. 2007. Biocontrol of *Rhizoctonia solani* in native potato (*Solanum phureja*) plants using native *Pseudomonas fluorescens*. Acta Biol. Colomb. 12, 19-32.

Beltrán, C., A. Cotes, and A. Paris. 2007. Selection of isolates of *Trichoderma* spp. with biocontrol activity over *Rhizoctonia solani* in potato. IOBC/WPRS Bull. 30, 55-58.

Campion, C., C. Chatot, B. Perraton, and D. Andrivon. 2003. Anastomosis groups, pathogenicity and sensitivity to fungicides of *Rhizoctonia solani* isolates collected on potato crops in France. Eur. J. Plant Pathol. 109, 983-992.

Campos, A.P.S. and P.C. Ceresini. 2006. Somatic incompatibility in *Rhizoctonia solani* AG-1 IA of soybean. Summa Phytopathol. 32, 247-254.

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

Carling, D.E. and R.H. Leiner. 1986. Isolation and characterization of *Rhizoctonia solani* and binucleate *R. solani*-like fungi from aereal stems and subterranean organs of potato plants. Phytopathology 76, 725-729.

Carling, D.E., R.H. Leiner, and P.C. Westphale. 1989. Symptoms, signs and yield reduction associated with *Rhizoctonia* disease of potato induced by tuberborne inoculum of *Rhizoctonia solani* AG-3. Amer. Potato J. 66, 693-701.

Carling, D.E. and R.H. Leiner. 1990. Virulence of isolates of *Rhizoctonia solani* AG-3 collected from potato plants and soil. Plant Dis. 74, 901-903.

Carling, D.E., S. Kuninaga, and K.A. Brainard. 2001. Hyphal anastomosis reactions, rDNA-internal transcribed spacer sequences, and virulence levels among subsets of *Rhizoctonia solani* anastomosis group-2 (AG-2) and AG-BI. Phytopathology 92, 43-50.

Carling, D.E., R.E. Baird, R.D. Gitaitis, K.A. Brainard, and S. Kuninaga. 2002. Characterization of AG-13 a newly reported anastomosis group of *Rhizoctonia solani*. Phytopathology 92, 893-899.

Castro, C., J.R. Davis, and M.V. Wiese. 1988. Quantitative estimation of *Rhizoctonia solani* AG-3 in soil. Phytopathology 78, 1287-1292.

Cedeño, L., C. Carrero, K. Quintero, Y. Araujo, H. Pino, and R. García. 2001. Identificación y virulencia de grupos de anastomosis de *Rhizoctonia solani* Kühn asociados con papa en Mérida, Venezuela. Interciencia 26, 296-300.

Ceresini, P.C., H.D. Shew, R.J. Vilgalys, and M.A. Cubeta. 2002. Genetic diversity of *Rhizoctonia solani* AG-3 from potato and tobacco in North Carolina. Mycologia 94, 437-449.

Cubeta, M.A. and R. Vilgalys. 1997. Population biology of the *Rhizoctonia solani* complex. Phytopathology 87, 480-484.

Demirci, E., C. Eken, and H. Zengin. 2002. First report of *Rhizoctonia solani* and binucleate *Rhizoctonia* from Johnson grass in Turkey. Plant Pathol. 51, 391.

Escande, A.R. and E. Echandi. 1991. Protection of potato from *Rhizoctonia* canker with binucleate *Rhizoctonia* fungi. Plant Pathol. 40, 197-202.

Espinal, C.F., H. Martínez, N. Pinzón, and C. Barrios. 2006. La cadena de la papa en Colombia. Una mirada global de su estructura y dinámica 1991-2005. Ministerio de Agricultura y Desarrollo Rural, Bogota.

González, D., M.A. Cubeta, and R. Vilgalys. 2006. Phylogenetic utility of indels within ribosomal DNA and [beta]-tubulin sequences from fungi in the *Rhizoctonia solani* species complex. Mol. Phylogenet. Evol. 40, 459-470.

Hall, T.A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucl. Acids Symp. 41, 95-98.

Hartill, W.F.T. 1989. Some effects of *Rhizoctonia solani* on growth and yield of potatoes. Potato Res. 32, 283-292.

ICA. 2003. Resolución 2501 por la cual se establecen los requisitos específicos mínimos para la producción de semilla certificada de papa. Bogota.

Jeger, M.J., G.A. Hide, P.H.J.F. Van Den Boogert, A.J. Termorshuizen, and P. Van Baarlen. 1996. Pathology and control of soil-borne fungal pathogens of potato. Potato Res. 39, 437-469.

Ko, W. and F. Hora. 1979. A selective medium for the quantitative determination of *Rhizoctonia solani* in soil. Phytopathology 61, 707-710.

Kuninaga, S., T. Natsuaki, T. Takeuchi, and R. Yokosawa. 1997. Sequence variation of the rDNA ITS regions within and between anastomosis groups in *Rhizoctonia solani*. Curr. Genet. 32, 237-243.

Lees, A.K., D.W. Cullen, L. Sullivan, and M.J. Nicolson. 2002. Development of conventional and quantitative real-time PCR assays for the detection and identification of *Rhizoctonia solani* AG-3 in potato and soil. Plant Pathol. 51, 293-302.

Lehtonen, M.J., P. Somervuo, and J.P.T. Valkonen. 2008. Infection with *Rhizoctonia solani* induces defense genes and systemic resistance in potato sprouts grown without light. Phytopathology 98, 1190-1198.

MacNish, G.C., D.E. Carling, and K.A. Brainard. 1997. Relationship of microscopic and macroscopic vegetative reactions in *Rhizoctonia solani* and the occurrence of vegetatively compatible populations (VCP) in AG-8. Mycol. Res. 101, 61-68.

Martin, B. 1988. Identification, isolation, frequency, and pathogenicity of anastomosis groups of binucleate *Rhizoctonia* spp. from strawberry roots. Phytopathology 78, 379-384.

McCabe, P.A., M.P. Gallagher, and J.W. Deacon. 1999. Microscopic observation of perfect hyphal fusion in *Rhizoctonia solani*. Mycol. Res. 103, 487-490.

Murashige, T. and F. Skoog. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. 15, 473-497.

Ogoshi, A. 1987. Ecology and pathogenicity of anastomosis and intraspecific groups of *Rhizoctonia Solani* Kuhn. Annu. Rev. Phytopathol. 25, 125-143.

Ogoshi, A. 1996. The genus *Rhizoctonia*. pp. 1-9. In: Sneh, B., S. Jabaji-Hare, S. Neate, and G. Dijst (eds.). *Rhizoctonia* species: taxonomy, molecular biology, ecology, pathology and disease control. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Platt, H.W., F. Canale, and G. Gimenez, 1993. Effects of tuber-borne inoculums of *Rhizoctonia solani* and fungicidal seed potato treatment of plant growth and *Rhizoctonia* disease in Canada and Uruguay. Amer. Potato J. 70, 553-559.

Poromarto, S.H., B.D. Nelson, and T.P. Freeman. 1998. Association of binucleate *Rhizoctonia* with soybean and mechanism of biocontrol of *Rhizoctonia solani*. Phytopathology 88, 1056-1067.

Rosa, D.D., E.E. Kuramae, R.C. Fenille, and N.L. Souza. 2005. Caracterização citomorforlógica, molecular e patogênica de isolados de *Rhizoctonia solani* na cultura da batata (*Solanum tuberosum*). Summa Phytopathol. 31, 133-141.

Scholte, K. 1989. Effects of soil-borne *Rhizoctonia solani* Kuhn on yield and quality of ten potato cultivars. Potato Res. 32, 367-376.

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

Silva-Barreto, F.A.S., W.V. Pereira, M.B. Ciampi, M.P.S. Câmara, and P.C. Ceresini. 2010. Associação de *Rhizoctonia solani* grupo de anastomose 4 (AG-4 HGI e HGIII) à espécies de plantas invasoras de área de cultivo de batata. Summa Phytopathol. 36, 145-154.

Sneh, B., L. Burpee, and A. Ogoshi. 1998. Identification of *Rhizoctonia* species. APS press, St. Paul, MN.

Truter, M. and F.C. Wehner. 2004. Anastomosis grouping of *Rhizoctonia solani* associated with black scurf and stem canker of potato in South Africa. Plant Dis. 88, 83.2-83.2.

Tsror, L. 2010. Biology, epidemiology and management of *Rhizoctonia solani* on potato. J. Phytopathol. 158, 649-658.

Tsror, L. and I. Peretz-Alon. 2005. The influence of the inoculum source of *Rhizoctonia solani* on development of black scurf on potato. J. Phytopathol. 153, 240-244.

Virgen-Calleros, G., V. Olalde-Portugal, and D.E. Carling. 2000. Anastomosis groups of *Rhizoctonia solani* on potato in central México and potential for biological and chemical control. Amer. J. Potato Res. 77, 219-224.

White, T.J., T. Burns, S. Lee, and J. Taylor. 1990. Amplification and direct sequencing of fungal ribosomal genes for phylogenetics. pp. 315-322. In: Innis, M.A., D.H. Gelfand, J. Shinsky, and T.J. White (eds.). PCR protocols. A guide to methods and applications. Academic Press, San Diego, CA.

Woodhall, J.W., A.K. Lees, S.G. Edwards, and P. Jenkinson. 2007. Characterization of *Rhizoctonia solani* from potato in Great Britain. Plant Pathol. 56, 286-295.

Woodhall, J.W., A.K. Lees, S.G. Edwards, and P. Jenkinson. 2008. Infection of potato by *Rhizoctonia solani*: effect of anastomosis group. Plant Pathol. 57, 897-905.