






Plant health and crop protection

Scientific and technological research article

Genetic diversity of *Rhizoctonia solani* AG-3PT, the etiological cause of stem canker and black scurf in Colombian potatoes

 Edisson Chavarro-Mesa^{1*},  Néstor Andrés Herrera-Blanco²,
 Camilo Rubén Beltrán-Acosta³,  Alba Marina Cotes-Prado³,
 Jorge Evelio Ángel-Díaz⁴

¹Universidad Tecnológica de Bolívar (UTB). Cartagena de Indias, Colombia.

²Servicio Nacional de Aprendizaje (SENA). Cali, Colombia.

³Corporación Colombiana de Investigación Agropecuaria (AGROSAVIA). C. I. Tibaitatá. Mosquera, Colombia.

⁴Instituto Colombiano Agropecuario (ICA). C.I. Tibaitatá, Mosquera, Colombia.

*Corresponding author: Universidad Tecnológica de Bolívar (UTB). Facultad de Ciencias Básicas, Campus Tecnológico, Parque Industrial y Tecnológico Carlos Vélez Pombo, Km 1 Vía Turbaco. Cartagena de Indias, Colombia. echavarro@utb.edu.co

Subject editor: John Fredy Hernández Nopsa (Corporación Colombiana de Investigación Agropecuaria [AGROSAVIA])

Received: March 25, 2020

Accepted: May 28, 2021

Published: October 18, 2021

How to cite this article: Chavarro-Mesa, E., Herrera-Blanco, N. E., Beltrán-Acosta, C. R., Cotes-Prado, A. M., & Ángel-Díaz, J. E. (2021). Genetic diversity of *Rhizoctonia solani* AG-3PT, the etiological cause of stem canker and black scurf in Colombian potatoes. *Ciencia y Tecnología Agropecuaria*, 22(3), e1888. https://doi.org/10.21930/rcta.vol22_num3_art:1888



Abstract

Stem canker and black scurf are potato diseases caused by the fungus *Rhizoctonia solani*, anastomosis group 3 (AG-3PT). This fungus affects potato roots, stems, and tubers, reducing crop yields. This study aims to determine the genetic diversity of *R. solani* AG-3PT obtained from the Colombian departments of Antioquia, Boyacá, and Cundinamarca. Restriction fragment length polymorphism (RFLP) analysis of the ITS-5.8S ribosomal DNA region allowed the differentiation and specific identification of AG-3PT and AG2-1 anastomosis groups. These results confirm that AG-3PT is the primary causative agent and etiological origin of stem canker and black scurf in Colombia. Two distinct subgroups within *R. solani* AG-3PT were identified using random amplified microsatellite markers (RAMs); AG-3PT (A) shares a similarity index of 78 %, and AG-3PT (B) shares a similarity of 79 % among its isolates. These subgroups were not linked to a geographical origin but to the AG group to which they belong. Nei's genetic diversity [D] of 0.25 confirmed the high genetic diversity for AG-3PT through RAMs analysis, related to a high evolutive potential within the AG-3PT group in Colombia. Finally, the *R. solani* AG-3PT fungus obtained from Cundinamarca had the adaptative potential to emerge as a pathogen of *Solanum phureja* in Colombia, possibly due to the similarity between pathosystems.

Keywords: random amplified microsatellites (RAMs), ribosomal DNA, *Solanum phureja*, *Solanum tuberosum*, *Thanatephorus cucumeris*

Diversidad genética de *Rhizoctonia solani* GA-3PT, causa etiológica del chancro del tallo y la sarna de la papa en Colombia

Resumen

El chancro del tallo y la sarna negra de la papa son enfermedades ocasionadas por el hongo *Rhizoctonia solani* grupo de anastomosis tres (GA-3PT), el cual afecta raíces, tallos y tubérculos de papa y reduce el rendimiento de los cultivos. El objetivo de la presente investigación fue determinar la diversidad genética de *R. solani* GA-3PT presente en los departamentos colombianos de Antioquia, Boyacá y Cundinamarca. La restricción enzimática de la región ribosomal (RFLP, por sus siglas en inglés) ITS-5.8S permitió la diferenciación e identificación específica de los grupos de anastomosis GA-3PT y GA2-1 y confirmó que el GA-3PT es el principal agente causal y origen etiológico de la enfermedad en Colombia. Mediante amplificación aleatoria de marcadores microsatélites (RAMs), por sus siglas en inglés, se observaron dos agrupamientos dentro de *R. solani* GA-3PT; el GA-3PT (A) comparte un índice de similitud del 78 % entre sí, en comparación con el GA-3PT (B) que presenta una similitud del 79 % entre sus aislamientos. Los agrupamientos no están relacionados con su origen geográfico, sino con el grupo de anastomosis al que pertenecen. La diversidad genética de Nei [D] de 0,25 confirma una alta diversidad genética para el GA-3PT mediante análisis RAMs, relacionada con un alto potencial evolutivo al interior del grupo GA-3PT en Colombia. Finalmente, el hongo *R. solani* GA-3PT que se obtuvo en Cundinamarca tiene potencial adaptativo para emerger como patógeno de la papa criolla (*Solanum phureja*) en Colombia, posiblemente, debido a la semejanza de los patosistemas.

Palabras clave: ADN ribosomal, amplificación aleatoria de microsatélites (RAMs), *Solanum phureja*, *Solanum tuberosum*, *Thanatephorus cucumeris*

Introduction

In Colombia, potato is one of the most important crops in cold temperature areas (Herrera et al., 2000). However, potato production is affected by *Rhizoctonia solani* AG-3PT, which is a natural inhabitant of soil (David et al., 2018). AG-3PT is the dominant anastomosis group (AG) most commonly associated with potatoes (Inokuti et al., 2019). *Rhizoctonia solani* is the causal agent of stem canker and black scurf diseases in Colombian potatoes (Ferrucho et al., 2012), causing necrotic lesions primarily in roots and stems (Aliferis & Jabaji, 2012; David et al., 2018). Consequently, *R. solani* AG 3PT diseases result in significant yield losses and reduced tuber quality (Beltrán et al., 2007; Muzhinji et al., 2017).

Rhizoctonia solani AG-3PT fungus survives in the soil as mycelia in decomposing tissues and sclerotia on infected tubers and soil (Rioux et al., 2011; Tsrer, 2010). Sclerotia adhere to the surface of potato tubers causing black scurf, a severe problem for crop production and distribution and for pathogen-free potato seed production and sale (Inokuti et al., 2019).

The availability of fast and specific diagnostic tests is a pressing need to understand plant pathogens' ecological, molecular genetics, and epidemiological aspects (Woodhall et al., 2013). The capacity to detect a pathogen and its anastomosis groups in crops would enable researchers to establish its inoculum levels, providing valuable information for decision making during plant disease management (Lees et al., 2002; Woodhall et al., 2013).

Genetic diversity and molecular phylogenetic studies on *Rhizoctonia* spp. have been limited to the characterization of anastomosis groups and primarily the differentiation between *Ceratobasidium* spp. and *R. solani* isolates (Roberts, 2000). Previous research conducted on *R. solani* had provided evidence of the genetic variability displayed by most anastomosis groups, in some cases suggesting the need to redefine the concept of this species (González-García et al., 2006; Roberts, 2000). Most molecular techniques are based on detecting and genotyping polymorphisms present in the genome at various levels (González-García et al., 2006; Helgason et al., 2003). For example, the analysis of genetic variations in ribosomal sequences helped to identify two phylogenetic groups within AG-3 that have been associated with potato (AG-3PT) and tobacco (AG-3TB) diseases (Kuninaga et al., 2000). Amplified Fragment Length Polymorphisms Analyses (AFLPs) were used to confirm this on isolates obtained from potatoes and tobacco, finding that AG-3PT isolates were not pathogenic to tobacco, and AG-3TB isolates were not pathogenic to potatoes (Ceresini, Shew, Vilgalys, & Cubeta, 2002). Elbakali et al. (2003) characterized *R. solani* isolates obtained from potatoes in Spain using Internal Transcribed Spacer Regions-Restriction Fragment Length Polymorphism (ITS-RFLP) and Random Amplified Microsatellites (RAMs); AG-3 was differentiated and separated from the other anastomosis groups. However, the RAMs analysis was better suited to genetic diversity studies (Hantula et al., 1996) since ITS-RFLP, which consists of monomorphic band patterns, only allowed the differentiation of the AG under study.

Recently, a genetic structure study on AG-3PT in Colombia showed a low differentiation level between populations, contemporaneous gene flow, and mixed reproduction with evidence of inbreeding, which is possibly associated with high evolutive potential within the studied isolates (Ferrucho et al., 2013). Therefore, it is necessary to understand i) the epidemiological implications of AG-3PT in Colombian

potato crops, which are described as the primary etiological agent of the diseases, and ii) the risk level and evolutive potential of the pathogen concerning its genetic diversity.

This study aims to determine the genetic diversity of *R. solani* AG-3PT present in the Colombian departments of Antioquia, Boyacá, and Cundinamarca, which are important areas in the potato production chain. Furthermore, we evaluated the pathogenicity of *R. solani* AG-3PT isolates on the potato species *Solanum tuberosum* and *S. phureja*, with *Phaseolus vulgaris* as a non-host control, to observe the range, specialization, and incipient adaptation of the fungus to other species. We concluded that the pathogen *R. solani* AG-3PT was not host-specific nor genetically isolated and, therefore, it can maintain a wide range of hosts.

Materials and methods

Field sampling and fungal isolation

Fifty isolates were obtained from symptomatic tubers on two potatoes cultivars (Diacol Capiro and Parda Pastusa) in Antioquia, Boyacá, and Cundinamarca (figure 1 and table 1). We sampled five rows per field; tuber samples were taken every 10 m from each row following a transept sampling method (McDonald, 1997).



Figure 1. Origin of *Rhizoctonia solani* isolates obtained from Antioquia, Boyacá, and Cundinamarca in Colombia.

Source: Elaborated by the authors

Table 1. Coordinates of the isolates

Department	Municipality	Host	AGs identified by molecular detection		Geographical coordinates
			<i>R. solani</i> AG-3PT	<i>R. solani</i> AG2-1	
Cundinamarca	Carmen de Carupa	<i>Solanum tuberosum</i>	15	1	05°21'01"N, 73°04'05"W
Boyacá	Ventaquemada	<i>Solanum tuberosum</i>	17	1	05°21'59"N, 73°31'19"W
Antioquia	La Unión	<i>Solanum tuberosum</i>	15	1	05°58'22"N, 75°21'40" W
Total AGs identified				N = 50	

Source: Elaborated by the authors

The isolates were in Alkaline Water Agar (AA) (BD Difco™, pH 8.5), and the fungal colonies characteristic of *Rhizoctonia* spp. were purified using Potato Dextrose Agar (PDA) (BD Difco™) and yeast dextrose- cultured bacto-peptone broth (YDB) (BD Difco™), acidified with gallic acid (0.3 g/L) (Beltrán et al., 2007). Sclerotia were transferred to Eppendorf tubes containing silica gel and stored at -20 °C to preserve the isolates; for non-sclerotic isolates, preservation was done in fungus-colonized rice grains, supplemented with 50 mg/L chloramphenicol (Chavarro-Mesa et al., 2015; Ciampi et al., 2008). The anastomosis or hyphal interaction compatibility tests were performed according to Carling et al. (1998) using UNAL AG-3 as the control strain (this group has the most significant incidence on potato crops in Colombia); it was supplied by the Agricultural Engineering School of Universidad Nacional de Colombia, Bogotá.

Characterization of diametral growth

We evaluated 26 isolates of *R. solani* AG-3, whose identification codes were RH006, RH015, RH020, RH025, RH027, RH029, RH030, RH033, RH034, RH035, RH040, RH042, RH044, RH054, RH066, RH069, RH075, RH080, RH099, RH106, RH110, RH120, RH140, RH176, RH200, and AG3-Unal. Five-millimeter discs between 4 to 5 days old were used as inoculum. Discs with the isolates were placed in PDA for incubation at 10, 20, and 25 °C in complete darkness; the assays were performed in triplicate for each isolate. The diametral growth (mm) was measured for the colonies at each temperature after ten days of incubation. Data were analyzed using ANOVA ($p \leq 0.05$) and a mean comparison test (Tukey). PAST 3.22 software ran the analysis (Hammer et al., 2001).

Genetic diversity analysis

We extracted the DNA using mycelial growth from PDA cultures, according to Goodwin and Lee (1993). Molecular identification of *Rhizoctonia* isolates was achieved by PCR (Polymerase Chain Reaction) tests based on selective amplification using primers specifically designed for *R. solani* AG-3PT: Rs1F2 (5'-

TTGGTTGTAGCTGGTCTATTT-3') and Rs2R1 (5'-TATCACGCTGAGTGGAACCA-3') (Lees et al., 2002). Genetic diversity analyses were carried out by amplifying the ITS-5.8S rDNA followed by a restriction with the endonucleases *AluI*, *EcoRI* (Fermentas[®]), and *TaqI* (Promega[®]). Also, RAMs assay was performed using primers (TCG [5'DHB (TCG)₅], GT [5'VHV (GT)₇G], and AC [5'DHD (AC)₇A]) (Elbakali et al., 2003).

The data obtained here were used to construct a genetic distance matrix based on Nei's method (Nei, 1973). To visualize the genetic differentiation between the *R. solani* group AG-3PT isolates, we made a dendrogram and a principal components analysis (PCA) by UPGMA using R programming environment (R Core Team, 2017). The compatibility of the cluster analysis with the original data was evaluated based on the cophenetic correlation coefficient. We assessed the consistency of the dendrogram nodes based on 10,000 bootstraps performed with the pvclust R package (Suzuki & Shimodaira, 2006). The discrimination capacity of the RAMs technique is mathematically defined as the probability that two isolates chosen randomly from a population of nonrelated isolates can be distinguished from one another (Hunter, 1990; Hunter & Gaston, 1988). The reproducibility of the RAMs technique was tested according to Pérez et al. (1998), evaluating the parameters of auto similarity, band repeatability, and the average frequency of each band.

Cross pathogenicity test

We selected four *R. solani* AG-3 PT isolates from *S. tuberosum* (RH001T, RH002T, RH004T, RH006T) and four isolates of *S. phureja* cultivar "yema de huevo" obtained in Cundinamarca in 2018 (RH001P, RH002P, RH004P, and RH005P) to perform cross pathogenicity tests on *S. phureja*, *S. tuberosum*, and *P. vulgaris*. *R. solani* AG-3PT inoculum was obtained from colonized parboiled rice and attached with parafilm to the base of the last or second-to-last leaf of the main stem in plants with four leaves (15 days after sowing). The inoculated plants were kept in a greenhouse with high humidity (95 %) and daytime temperatures between 15 and 20 °C. An evaluation was performed 15 days after the inoculation by measuring the lesions on leaves and stems. The disease index was calculated according to three severity scales described by Horsfall and Barratt (1945), Jia et al. (2007), and Salazar-Zuluaga (2014).

The experimental design employed was completely randomized blocks with eight treatments and four replicates and one repetition of the entire experiment in time. The mean disease severity for the groups of isolates followed by the same letter (figure 7) is not significantly different according to a contrast of means at $p \leq 0.05$ (Chavarro-Mesa et al., 2015, 2020; Ramos-Molina et al., 2016). The rating of the experimental unit involved the combined scores from the four plants. Linear and independent ANOVA models were created for each evaluation parameter (Chavarro-Mesa et al., 2015; Ramos-Molina et al., 2016) using the R programming environment (R Core Team, 2017) to detect evidence of host specialization.

Results and discussion

Fungal isolation and growth characterization

The occurrence of *R. solani* AG-3PT infection in potato crops found in this study correlates with previous reports in Colombia (Ferrucho et al., 2012) and worldwide (Das et al., 2014; Muzhinji et al., 2015; Tsrer, 2010; Yang et al., 2015, 2017). Regarding macroscopic characteristics of *R. solani*, the isolates developed the usual white/colorless mycelial growth during the early stages, turning light brown after reaching maturity (figures 2A and 2B) (Ceresini et al., 1999). In addition, we observed microscopic characteristics such as 90° angles formed by branching hyphae of septate mycelium (figure 2C) (Ajayi-Oyetunde & Bradley, 2017; Ceresini et al., 1999) and hyphae clumps originating sclerotia (figure 2D) (Ajayi-Oyetunde & Bradley, 2017). Furthermore, the multinucleated state of the fungus was also determined, as was anastomosis type C3 (Carling et al., 1998), because hyphae displayed a perfect fusion between *R. solani* AG-3PT hyphae (figures 2E and 2F) (Ajayi-Oyetunde & Bradley, 2017).

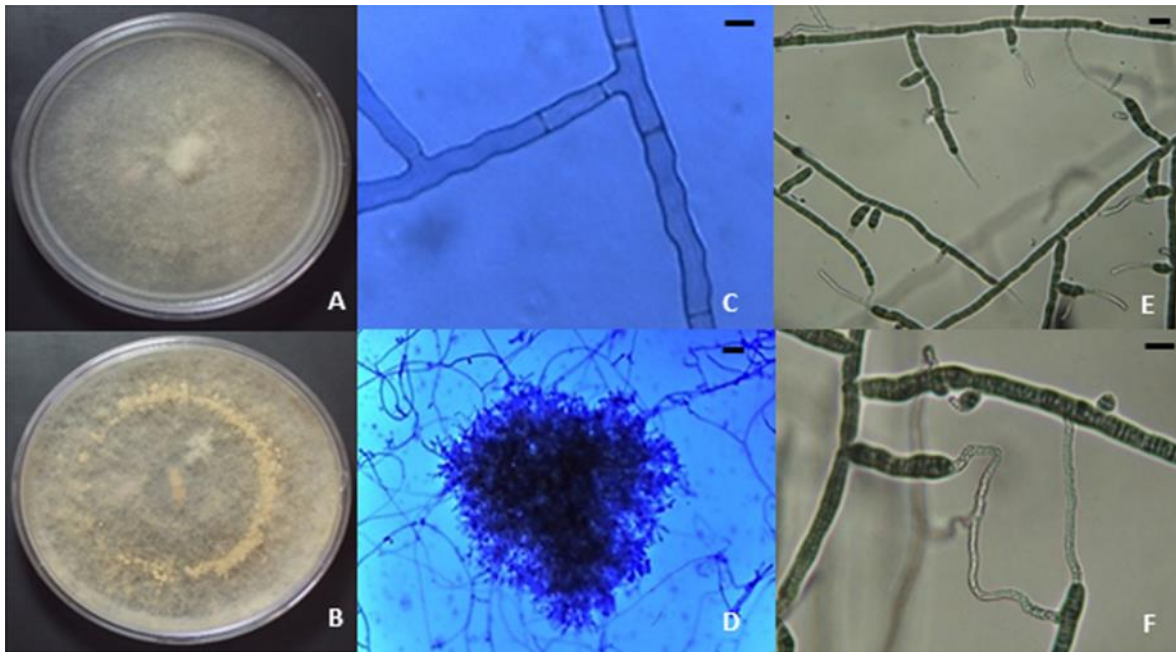


Figure 2. Macro and microscopic characteristics of *Rhizoctonia solani*. A) *R. solani* AG2-1; B) *R. solani* AG-3; C) 90° angled branching of septate hyphae, 40x; D) monilloid cells of sclerotia, 10x; E) hyphal anastomosis reaction for the anastomosis group designation of *R. solani* AG-3 isolates, 40x; F) Hyphal anastomosis and multinucleate *R. solani*, 40x. Bars = 10 µm.

Photos: Edisson Chavarro-Mesa, Néstor Andrés Herrera-Blanco, Camilo Rubén Beltrán-Acosta

The AG-3 group of *R. solani* was present in the three evaluated regions (figure 1), as observed by Carling et al. (2002), Cedeño et al. (2001), and Ferrucho et al. (2012), who established the AG-3PT group as the most common and widespread and the leading cause of potato rhizoctoniosis in Colombia. Color diversity among the colonies was also noted; they ranged between light yellow, light brown, and dark

brown (figure 3B). Additionally, there were differences in the size, shape, and color of the sclerotia and the distribution of the culture medium (figure 3B).

About the growth characterization of *R. solani* (figure 3A), all isolates showed the highest growth at 20 and 25 °C. On the sixth day, the growth was between 65 and 85 mm at 20 °C, and between 73 and 85 mm at 25 °C. In contrast, at 10 °C, 75 % of the evaluated isolates showed diameters between 30 and 50 mm after ten days of incubation. The remaining 25 % had colony diameters between 51 and 75 mm (figure 3A). Notice that the development of sclerotia was favored at 25 °C (figure 3B). The presence of AG-3PT is related to environmental conditions such as temperature, prevailing in cold climates (Anguiz & Martin, 1989; Campion et al., 2003).

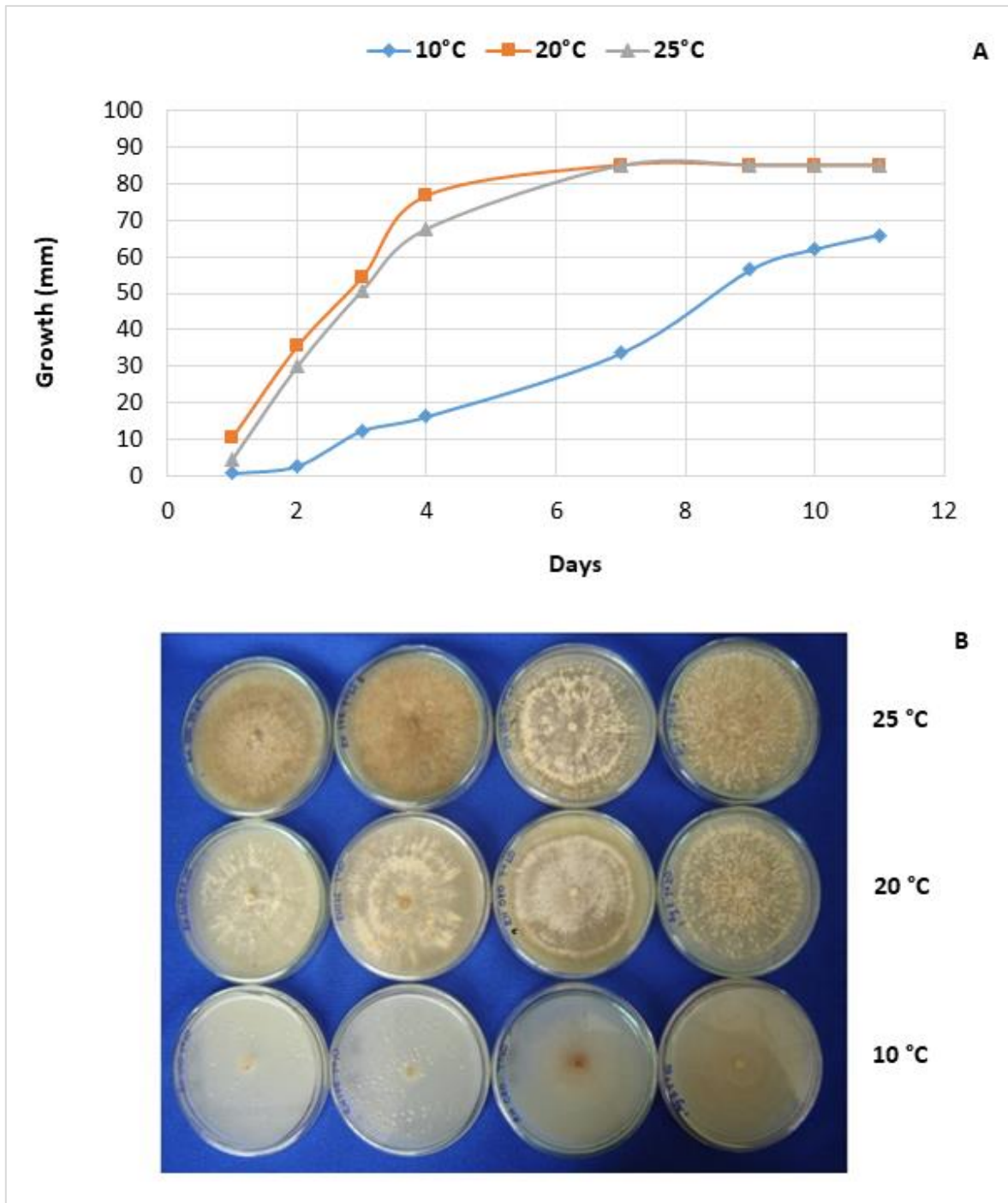


Figure 3. Diametral growth of *R. solani* mycelium AG-3 at 10, 20, and 25 °C. A) Mycelial growth relative to 26 isolations, mean of six repetitions (combination of two independent experiments with three replicates each); B) macroscopic characterization of *R. solani* at three different temperatures (10, 20, and 25 °C), colonies' color diversity.

Source: Elaborated by the authors. Photos taken by Edison Chavarro-Mesa, Néstor Andrés Herrera-Blanco, Camilo Rubén Beltrán-Acosta

Genetic diversity

The molecular diagnostics methods allowed identifying *R. solani* AG-3PT and *R. solani* AG2-1 (figure 1) from the 50 analyzed isolates, which were further differentiated using species-specific markers (Lees et al., 2002; Woodhall et al., 2013). *Rhizoctonia solani* is a pathogen that can survive on tubers and soil; thus, the detection of inoculum levels on tuber seeds and soil becomes relevant when attempting to predict the potential damage to farming and nonfarming areas, and it is an essential factor in managing the disease (Tsrör, 2010). It must be highlighted that the phytopathogenic fungus *R. solani* is often transmitted through contaminated tubers that are eventually used as seeds, which can establish a long-distance dispersion mechanism and infect pathogen-free regions. Once established in the soil, the mycelia and sclerotia of the microorganism provide a continuous source of primary inoculum (Tsrör, 2010).

With the development of specific and sensitive techniques, such as PCR and qPCR to detect *R. solani* AG-3PT, it is now possible to perform epidemiological studies in Colombian potatoes crops (Lees et al., 2002; Woodhall et al., 2013). The restriction analysis of the ITS-5.8S rDNA region using the endonuclease *EcoRI* in two anastomosis groups (AG-3PT and AG2-1) allowed the specific identification of each group from isolates collected in Antioquia, Boyacá, and Cundinamarca, revealing different band patterns between AG-3PT and AG2-1 (figure 4A).

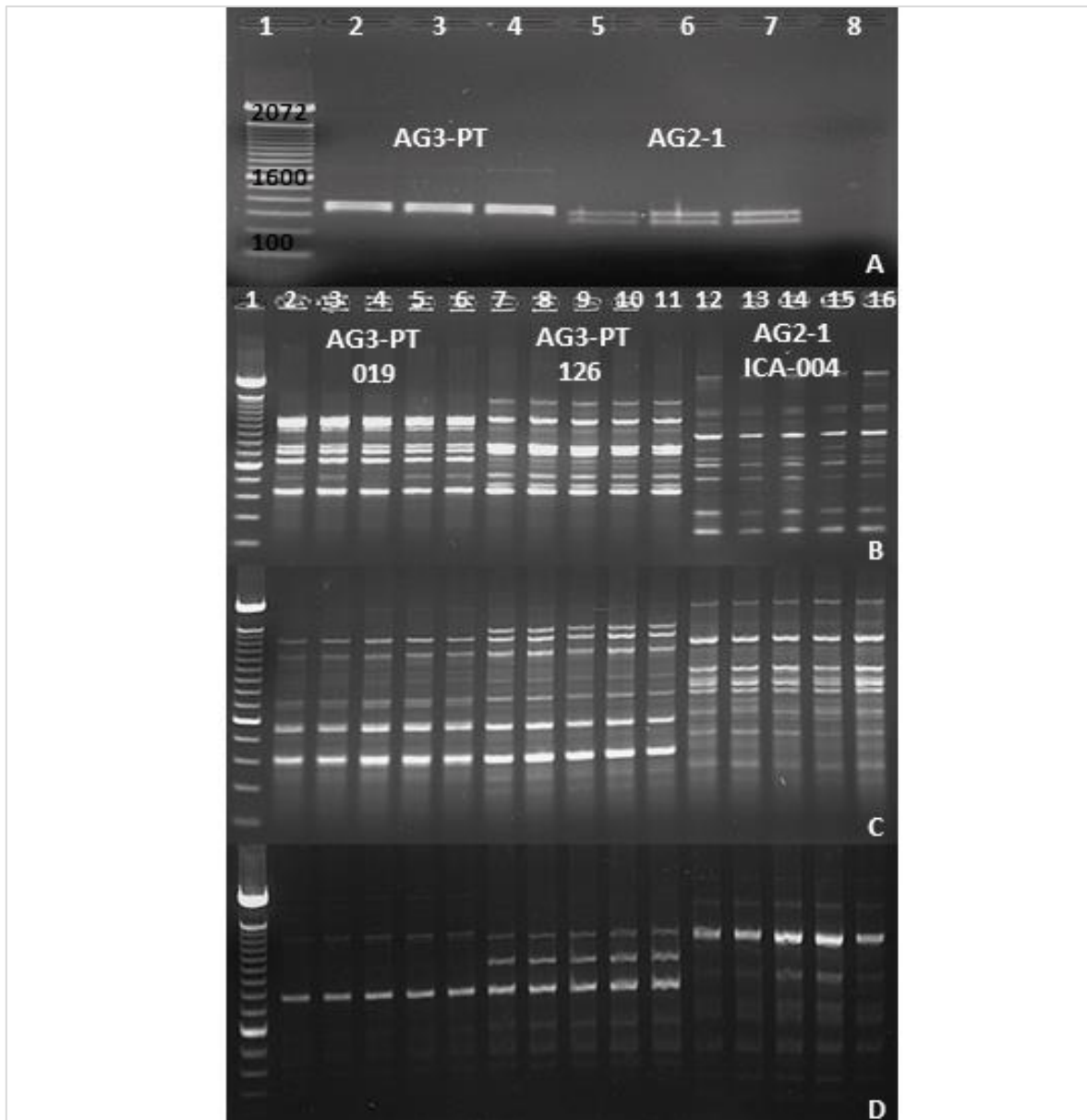


Figure 4. Identification and differentiation of AG-3 PT and AG2-1 using molecular markers. A) Restriction analysis with endonuclease *EcoRI* targeting the ITS-5.8S region in AG-3PT and AG2-1 isolates. Lane 1, 100 bp molecular weight marker (Invitrogen®); lanes 2-4, *R. solani* AG-3PT isolates; lanes 5-7, *R. solani* AG2-1 isolates; lane 8, PCR negative control. B), C), and D) Profile differentiation of AG-3 PT and AG2-1 isolates using AC, GT, and TCG primers (RAMs). Lane 1, 100 bp molecular weight marker (Invitrogen®); lanes 2-6, five replicates of the AG-3PT isolate (019); lanes 7-11, five replicates of the AG-3PT isolate (126); lanes 12-16, five replicates of the AG2-1 isolate (ICA 004). Positive and negative controls were included in each run of PCR-RAMs reactions.

Source: Elaborated by the authors. Photos taken by Edison Chavarro-Mesa, Néstor Andrés Herrera-Blanco, Camilo Rubén Beltrán-Acosta

The genetic diversity of the *R. solani* AG-3PT isolates also revealed polymorphic RAMs band patterns (figures 4B to 4D). The resulting dendrograms (figure 4A) showed two *R. solani* AG-3PT subgroups that (A) share a similarity index of 78 % in comparison to AG-3PT, and (B) bear a similarity of 79 % among isolates. Besides, the isolates 056A and ICA 004C (AG2-1) showed a similarity of 34 % when compared to the AG-3PT isolates (figures 5A and 5B). The primers AC and GT produced a single monomorphic band in all isolates, resulting in 92.3 and 91.6 % polymorphic loci. These values were higher than those obtained with the TCG primer, which resulted in only three polymorphic bands (42.8 % of polymorphic loci). The average genetic diversity values of *R. solani* AG-3PT were estimated to be 0.15, 0.27, and 0.31 for each primer set (TCG, AC, and GT, respectively); in turn, the global average (Hj) for Nei's genetic diversity was estimated to be 0.25.

Furthermore, the isolate groups evaluated through *R. solani* AG-3PT dendrograms using RAMs did not show any geographical relation between them (figures 5A and 5B). The latest suggests a clonal expansion and dispersion of evolutionarily adapted clones within *R. solani* AG-3PT populations. Previously, Ceresini, Shew, Vilgalys, Rosewich, & Cubeta (2002) found evidence of *R. solani* AG-3PT clonal isolation and recombination in North Carolina, USA, through AFLP analysis. Additionally, Justesen et al. (2003) used ITS sequencing and random amplified polymorphic DNA (RAPD) analyses to find clonal isolates of AG-3PT in Danish potato crops. Other studies consider this clonal trait a result of its evolutive potential (Mandujano et al., 2001; Mogie & Hutchings, 1990). Interestingly, this clonal capacity can be observed as an ecological alternative that enables the fungus to favor the dispersion of a specific genotype through space and time (Mandujano et al., 1998, 2001).

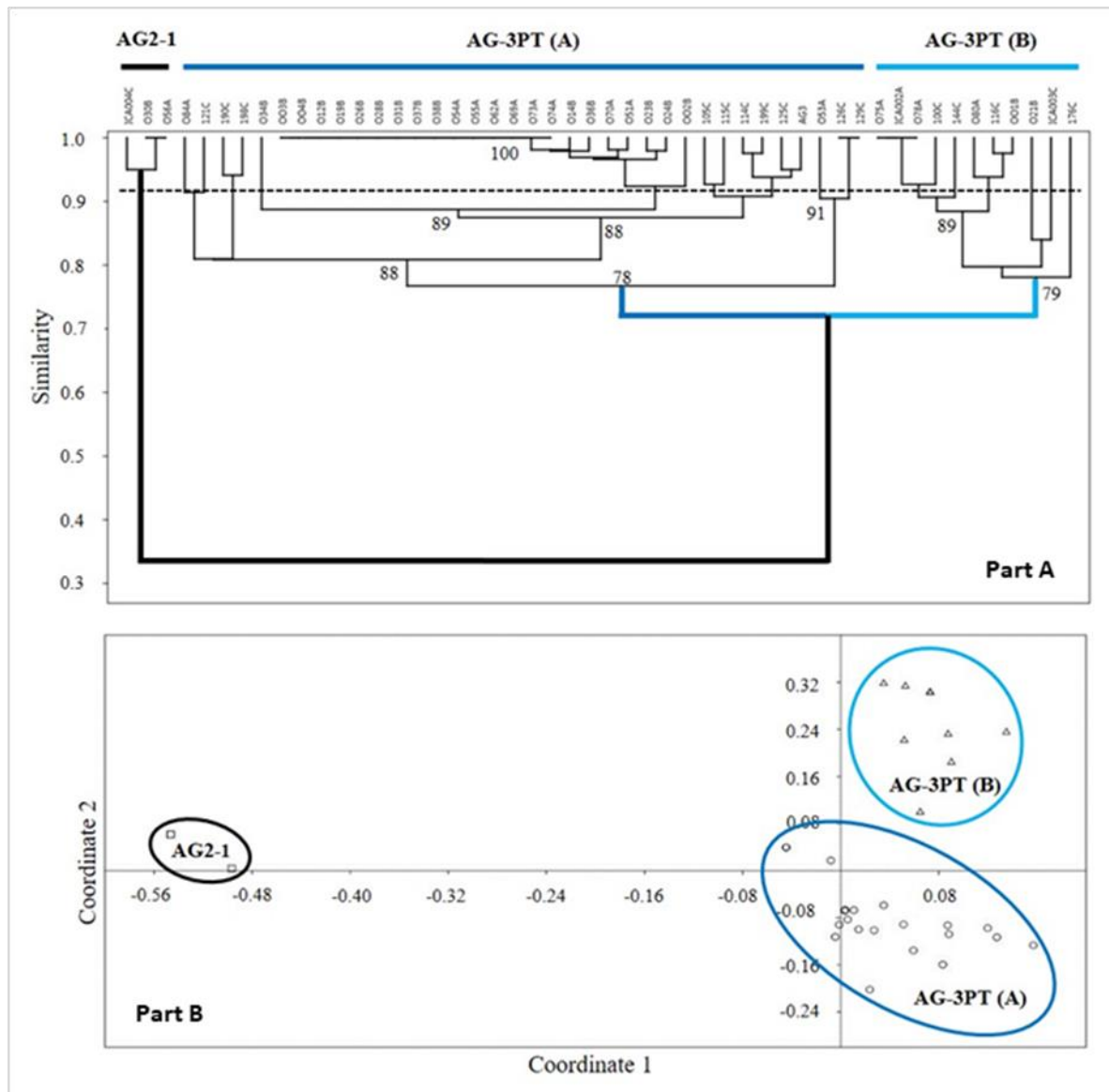


Figure 5. RAMs analysis: Part A) UPGMA similarity tree for the *R. solani* AG-3PT PCR-RAMs amplicons using standardized Nei's method; letters followed by the isolation code correspond to A = Antioquia, B = Boyacá, and C = Cundinamarca. Node numbers represent the resampling analysis support value. The horizontal dotted line represents the cutoff estimated for the AG-3PT group isolates. Part B) Principal component analysis based on the similarity indexes of RAMs patterns for *R. solani* AG-3PT, reconfirming the observations from the dendrogram tree.

Source: Elaborated by the authors

Our results revealed similarities of 78 and 100 % between AG-3PT isolates, suggesting that RAMs analysis is a suitable tool for evaluating the genetic diversity of a given population. These markers target sequence changes in microsatellites and can therefore be used to perform a complete genome analysis, facilitating the detection of polymorphisms. Interestingly, the analysis of AG2-1 isolates revealed a similarity percentage of 34 % compared to the entire AG-3PT population. The low similarity value

between AGs demonstrates that in comparison with other molecular techniques, RAMs detect genetic dissimilarities between AGs (Elbakali et al., 2003; Justesen et al., 2003) (figures 4B and 5A-B).

Nei's genetic diversity value observed in this study was 0.25, similar to values from previous reports of 0.28 to 0.38 (Ceresini, Shew, Vilgalys, & Cubeta, 2002) and 0.29 (Justesen et al., 2003). Recently, Muzhinji et al. (2017) reported that South African populations of *R. solani* AG-3PT are genetically differentiated, with Nei's genetic diversity values from 0.24 to 0.27. High genetic and genotypic diversity combined with a mixed reproduction capacity and high genetic flow between genotypes within populations are characteristic traits of phytopathogens with high evolutive potential (McDonald & Linde, 2002; Willi et al., 2011).

Recent studies demonstrated that like anastomosis group AG-1 IA, the AG-3PT group possesses a high evolutive potential; this phenomenon requires special attention regarding the proper planning of control strategies and disease management. In addition, the prolonged application of fungicides results in long-lasting resistance periods within *R. solani* populations (Chavarro-Mesa et al., 2015; Ferrucho et al., 2013; Gonzalez-Vera et al., 2010), and therefore, its integral management through biological control strategies could be a viable alternative for the phytosanitary maintenance of potato cultivars in Colombia (Beltrán et al., 2011; Cotes, 2018).

The discriminatory capacity of RAMs in the present study was 94 %, whereas the average auto similarity (S_{xx}) and average band repeatability (Rb) values were between 0.96 and 1.00. The average band frequency (fb) showed values between 0.54 and 0.56, probably because the AG-3PT and AG2-1 isolates share few loci (Hunter, 1990; Hunter & Gaston, 1988). RAMs are reproducible over time, and a lack of prior knowledge of the genome under evaluation demonstrates its efficiency at the rapid identification of AGs within the *R. solani* complex. Also, this same technique can be used in the genetic differentiation of isolates within the same species. AGs have been compared using DNA/DNA hybridization, RFLPs, RAPD, RAMs, isoenzyme electrophoresis, pectic zymograms, and DNA restriction mapping in the rDNA ITS 5.8S region, all of which indicate that the different AGs and subgroups are discrete evolutionary units (Balali et al., 1996; Carling et al., 2002; Elbakali et al., 2003; Kuninaga et al., 2000, 2002; Sharon et al., 2006).

The importance of genetic diversity analyses is directly related to the capacity of a species to survive variations in their environment, primarily because higher genetic diversity represents an increased probability of adapting to these continuous variations. However, species with low genetic diversity face a greater risk against environmental variations, impacting their population and increasing the rate of inbreeding, which further reduces the genetic diversity and makes the species vulnerable to extinction (Ramanatha & Hodgkin, 2002). Concerning phytopathogenic populations, genetic diversity is primarily related to its evolutive potential and population structure. High-risk pathogens that impact agricultural endeavors have high levels of genetic diversity and mixed reproduction systems (sexual/asexual), allowing the emergence and permanence of “new” pathogens in the agroecosystems (Chavarro-Mesa et al., 2015;

McDonald & Linde, 2002; Pereira et al., 2016; Willi et al., 2011). Moreover, this pathogen displays a highly conserved evolutive potential subject to the population structure of the fungus observed in Colombia, which could impact the management strategy and alternatives for this disease.

Pathogenicity assays

Symptoms present on different hosts (*S. tuberosum* and *S. phureja*) corresponded to the common symptoms of *R. solani* (figures 6A and 6B), such as stem canker and aerial blight of leaves. Pathogenicity was determined by estimating the lesion size on stems and leaves; in some cases, isolates showed distinct host ranges.



Figure 6. Pathogenicity test of *Rhizoctonia solani* group AG-3PT isolates in two different hosts (*Solanum phureja* and *S. tuberosum*). A) *R. solani* group AG-3PT isolates from *S. phureja*; B) *R. solani* group AG-3PT isolates from *S. tuberosum*.

Photos: Edisson Chavarro-Mesa, Néstor Andrés Herrera-Blanco, Camilo Rubén Beltrán-Acosta

Our results showed that the adaptation of *R. solani* AG-3PT to *S. tuberosum* promoted the appearance of symptoms in other potential hosts, such as *S. phureja* (figure 6). These fungal isolates, which were in the

initial stages of specialization, were not yet genetically isolated and therefore maintained a potentially wide range of hosts.

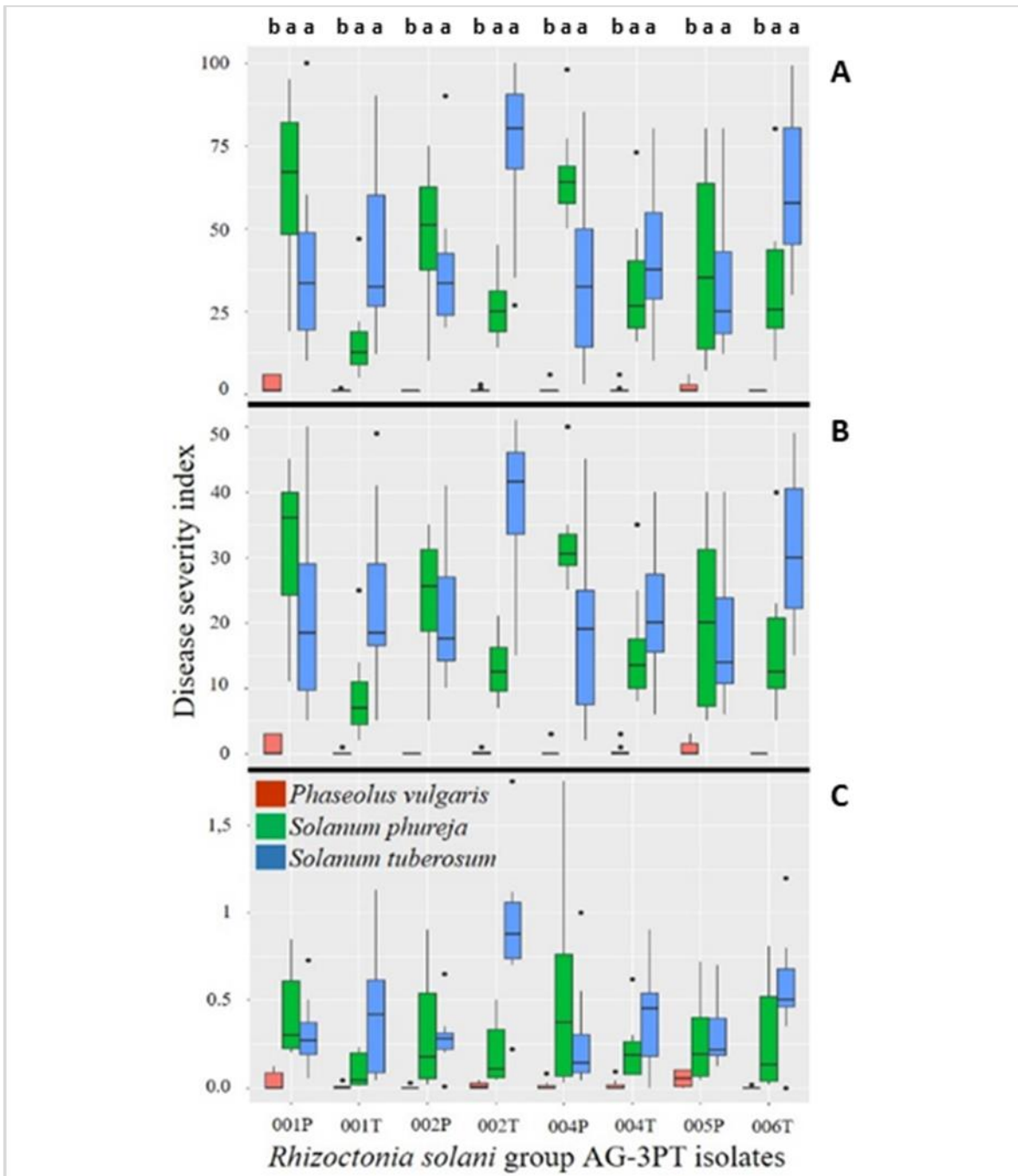


Figure 7. Cross pathogenicity tests of *R. solani* anastomosis group-3 PT isolates from *S. phureja* (RH001P, RH002P, RH004, RH005P) and *S. tuberosum* (RH001T, RH002T, RH004T, RH006T) in Cundinamarca, Colombia. Three different severity scales were used to evaluate the plant pathogen disease: A) Horsfall & Barratt (1945), B) Salazar-Zuluaga (2014), and C) Jia et al. (2007). *Phaseolus vulgaris* was not a host of *R. solani* group-3 PT. *S. phureja*, *S. tuberosum*, and *P. vulgaris* experiments were conducted independently. Source: Elaborated by the authors

Although many *R. solani* AGs are host-specific, some isolates have shown damage in different plants from their original host (Anderson, 1982; Ogoshi, 1987). Results described by Ferrucho et al. (2012) showed that *R. solani* AG-3 infected potatoes, tomatoes, and “lulo” or “naranjilla” (*Solanum quitoense*), exhibiting stem cankers; also, peas, beans, corn, and carrot showed root infection with the fungi. Implementation of crop rotation systems should consider non-hosts of AG-2-1 and AG-3PT. Interspecific adaptation events have been observed in other AGs; recently, it was determined that *R. solani* AG-1 IA isolates from *Urochloa* spp. in Colombia had the adaptive potential to become a pathogen in corn. These isolates from signal grass were more aggressive to the same host than the isolates from soybean, suggesting incipient host specialization (Poloni et al., 2016). Another study showed that signal grass isolates of *R. solani* AG-1 IA were more aggressive to *Urochloa brizantha* “Toledo” than those from rice (Chavarro-Mesa et al., 2015). Finally, *R. solani* AG-3PT isolates from Cundinamarca feature the adaptive potential to emerge as a pathogen in Andean potatoes (*S. phureja*) in Colombia, probably because of the similarity between pathosystems (figures 6 and 7).

Conclusions

We isolated and characterized 50 isolates from different crops in three Colombian regions, finding that *R. solani* AG-3PT was the most frequently fungi associated with potato cultivars. Growth characterization showed that all temperatures were adequate for the growth of the phytopathogen; however, 25 °C seemed to be the most favorable *in vitro*. Also, our molecular characterization employing different markers reaffirmed that this pathogen has a highly conserved evolutive potential associated with the genetic population structure found in Colombia. In combination with the pathogenicity tests, this characterization indicated the potential of this fungi to be an incipient pathogen in Andean potatoes (*S. phureja*).

Acknowledgments

This research was supported financially by the Department of Agriculture and Rural Development of Colombia (MADR), the Colombian Corporation for Agricultural Research (AGROSAVIA), and the Colombian Agricultural Institute (ICA). Many thanks to the anonymous reviewers who helped improve the manuscript.

Disclaimers

The authors declare no conflicts of interest.

References

- Ajayi-Oyetunde, O. O., & Bradley, C. A. (2017). Identification and Characterization of *Rhizoctonia* Species Associated with Soybean Seedling Disease. *Plant Disease*, 101(4), 520-533. <https://dx.doi.org/10.1094/pdis-06-16-0810-re>
- Aliferis, K. A., & Jabaji, S. (2012). FT-ICR/MS and GC-EI/MS metabolomics networking unravels global potato sprout's responses to *Rhizoctonia solani* infection. *PLoS ONE*, 7(8). <https://dx.doi.org/10.1371/journal.pone.0042576>
- Anderson, N. A. (1982). The Genetics and Pathology of *Rhizoctonia Solani*. *Annual Review of Phytopathology*, 20, 329-347. <https://dx.doi.org/10.1146/annurev.py.20.090182.001553>
- Anguiz, R., & Martin, C. (1989). Anastomosis groups, pathogenicity, and other characteristics of *Rhizoctonia solani* isolated from potatoes in Peru. *Plant Disease*, 73(3), 199-201. <https://doi.org/10.1094/pd-73-0199>
- Balali, G. R., Whisson, D. L., Scott, E. S., & Neate, S. M. (1996). DNA fingerprinting probe specific to isolates of *Rhizoctonia solani* AG-3. *Mycological Research*, 100(4), 467-470. [https://dx.doi.org/10.1016/S0953-7562\(96\)80145-6](https://dx.doi.org/10.1016/S0953-7562(96)80145-6)
- Horsfall, J. G., & Barratt, R. W. (1945). An improved grading system for measuring plant disease. *Phytopathology*, 35, 655. <http://www.garfield.library.upenn.edu/classics1986/A1986A666500001.pdf>
- Beltrán, C., Cotes, A. M., & Becerra, A. P. (2007). Selection of isolates of *Trichoderma* spp. with biocontrol activity over *Rhizoctonia solani* in potato. *IOBC WPRS Bulletin*, 30(6/2), 55-58. http://www.iobc-wprs.org/pub/bulletins/iobc-wprs_bulletin_2007_30_06.pdf
- Beltrán, C., Velandia, C., & Cotes, A. (2011). *Trichoderma koningiopsis* Th003, a biological alternative in the control of *Rhizoctonia solani* in potato cultivars. CORPOICA.
- Campion, C., Chatot, C., Perraton, B., & Andrivon, D. (2003). Anastomosis groups, pathogenicity and sensitivity to fungicides of *Rhizoctonia solani* isolates collected on potato crops in France. *European Journal of Plant Pathology*, 109(9), 983-992. <https://doi.org/10.1023/B:EJPP.0000003829.83671.8f>
- Carling, D. E., Brainard, K. A., Virgen-Calleros, G., & Olalde-Portugal, V. (1998). First Report of *Rhizoctonia solani* AG-7 on Potato in Mexico. *Plant Disease*, 82(1), 127. <https://dx.doi.org/10.1094/pdis.1998.82.1.127c>
- Carling, D. E., Kuninaga, S., & Brainard, K. A. (2002). 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(1), 43-50. <https://dx.doi.org/10.1094/PHYTO.2002.92.1.43>
- Cedeño, L., Carrero, C., Quintero, K., Araujo, Y., Pino, H., & García, R. (2001). Identificación y virulencia de grupos de anastomosis de *Rhizoctonia solani* Kühn asociados con papa en Mérida, Venezuela. *Interciencia*, 26(7), 296-300. <https://www.redalyc.org/pdf/339/33905805.pdf>
- Ceresini, P. C., Shew, H. D., & Cubeta, M. A. (1999). RFLP analysis of the PCR amplified ribosomal DNA regions ITS and IGS indicated that isolates of *Rhizoctonia solani* from potato and tobacco represent distinct groups within the anastomosis group 3. *Phytopathology*, 89, S12.
- Ceresini, P. C., Shew, H. D., Vilgalys, R. J., & Cubeta, M. A. (2002). Genetic diversity of *Rhizoctonia solani* AG-3 from potato and tobacco in North Carolina. *Mycologia*, 94(3), 437-449. <https://dx.doi.org/10.1080/15572536.2003.11833209>

- Ceresini, P. C., Shew, H. D., Vilgalys, R. J., Rosewich, U. L., & Cubeta, M. A. (2002). Genetic structure of populations of *Rhizoctonia solani* AG-3 on potato in eastern North Carolina. *Mycologia*, *94*(3), 450-460. <https://dx.doi.org/10.1080/15572536.2003.11833210>
- Chavarro-Mesa, E., Ceresini, P. C., Ramos Molina, L. M., Pereira, D. A. S., Schurt, D. A., Vieira, J. R., & McDonald, B. A. (2015). The *Urochloa* foliar blight and collar rot pathogen *Rhizoctonia solani* AG-1 IA emerged in South America via a host shift from rice. *Phytopathology*, *105*(11), 1475-1486. <https://dx.doi.org/10.1094/phyto-04-15-0093-r>
- Chavarro-Mesa, E., Ceresini, P., Pereira, D., Vicentini, S., Silva, T., Ramos-Molina, L., Negrisoli, M., Schurt, D., & Vieira Júnior, J. R. (2020). A broad diversity survey of *Rhizoctonia* species from the Brazilian Amazon reveals the prevalence of *R. solani* AG-1 IA on signal grass and the new record of AG-1 IF on cowpea and soybeans. *Plant Pathology*, *69*(3), 455-466. <https://dx.doi.org/10.1111/ppa.13142>
- Ciampi, M. B., Meyer, M. C., Costa, M. J. N., Zala, M., McDonald, B. A., & Ceresini, P. C. (2008). Genetic Structure of Populations of *Rhizoctonia solani* Anastomosis Group-1 IA from Soybean in Brazil. *Phytopathology*, *98*(8), 932-941. <https://dx.doi.org/10.1094/phyto-98-8-0932>
- Cotes, A. M. (Ed.). (2018). *Control biológico de fitopatógenos, insectos y ácaros* (Vol. 2). Corporación Colombiana de Investigación Agropecuaria (Agrosavia).
- Das, S., Shah, F. A., Butler, R. C., Falloon, R. E., Stewart, A., Raikar, S., & Pitman, A. R. (2014). Genetic variability and pathogenicity of *Rhizoctonia solani* associated with black scurf of potato in New Zealand. *Plant Pathology*, *63*(3), 651-666. <https://dx.doi.org/10.1111/ppa.12139>
- David, G. Q., Chavarro-Mesa, E., Schurt, D. A., & Ceresini, P. C. (2018). *Rhizoctonia* como fitopatógeno no agroecossistema brasileiro. In U.P. Lopes, & S.J. Michereff (Eds.), *Desafios Manejo Doenças Radiculares causadas por fungos* (pp. 33-55). <https://ainfo.cnptia.embrapa.br/digital/bitstream/item/178987/1/Livro-Desafios-Manejo-Doencas-Radiculares2.pdf>
- Elbakali, A. M., Lilja, A., Hantula, J., & Martin, M. P. (2003). Identification of Spanish isolates of *Rhizoctonia solani* from potato by anastomosis grouping, ITS-RFLP and RAMS-fingerprinting. *Phytopathologia Mediterranea*, *42*(2), 167-176. http://digital.csic.es/bitstream/10261/45785/1/2003_MPMartin_Phytopathol.Medit.%2042.pdf
- Ferrucho, R. L., Ceresini, P. C., Ramirez-Escobar, U. M., McDonald, B. A., Cubeta, M. A., & García-Domínguez, C. (2013). The population genetic structure of *Rhizoctonia solani* AG-3PT from potato in the Colombian Andes. *Phytopathology*, *103*(8), 862-869. <https://dx.doi.org/10.1094/PHYTO-11-12-0278-R>
- Ferrucho, R. L., Cifuentes, J. M., Ceresini, P., & García-domínguez, C. (2012). *Rhizoctonia solani* AG-3PT is the major pathogen associated with potato stem canker and black scurf in Colombia. *Agronomía Colombiana*, *30*(2), 204-2013. http://www.scielo.org.co/scielo.php?pid=S0120-99652012000200007&script=sci_arttext&tlng=en
- González-García, V., Portal Onco, M. A., & Rubio Susan, V. (2006). Review. Biology and systematics of the form genus *Rhizoctonia*. *Spanish Journal of Agricultural Research*, *4*(1), 55-79. <https://dx.doi.org/10.5424/sjar/2006041-178>
- Gonzalez-Vera, A. D., Bernardes-de-Assis, J., Zala, M., McDonald, B. A., Correa-Victoria, F., Graterol-Matute, E. J., & Ceresini, P. C. (2010). Divergence between sympatric rice-and maize-infecting populations of *Rhizoctonia solani* AG-1 IA from Latin America. *Phytopathology*, *100*(2), 172-182. <https://dx.doi.org/10.1094/PHYTO-100-2-0172>

- Goodwin, D. C., & Lee, S. B. (1993). Microwave miniprep of total genomic DNA from fungi, plants, protists and animals for PCR. *BioTechniques*, 15(3), 438-444. <http://europepmc.org/abstract/MED/8217156>
- Hammer, Ø., Harper, D., & Ryan, P. (2001). Past: Paleontological statistics software package for education and data analysis. *Paleontologia Electronica*, 4(1), 1-9. https://palaeo-electronica.org/2001_1/past/past.pdf
- Hantula, J., Dusabenyagasani, M., & Hamelin, R. C. (1996). Random amplified microsatellites (RAMS)-a novel method for characterizing genetic variation within fungi. *European Journal of Forest Pathology*, 26(3), 159-166. <https://dx.doi.org/10.1111/j.1439-0329.1996.tb00720.x>
- Helgason, T., Watson, I. J., & Young, J. P. W. (2003). Phylogeny of the Glomerales and Diversisporales (Fungi: Glomeromycota) from actin and elongation factor 1-alpha sequences. *FEMS Microbiology Letters*, 229(1), 127-132. [https://dx.doi.org/10.1016/S0378-1097\(03\)00802-4](https://dx.doi.org/10.1016/S0378-1097(03)00802-4)
- Herrera, C. A., Fierro, L. H., & Moreno, J. D. (Eds.). (2000). *Manejo integrado del cultivo de la papa* [Technical manual]. Corporación Colombiana de Investigación Agropecuaria (CORPOICA). <https://repository.agrosavia.co/handle/20.500.12324/34707>
- Hunter, P. R. (1990). Reproducibility and indices of discriminatory power of microbial typing methods. *Journal of Clinical Microbiology*, 28(9), 1903-1905. <https://doi.org/10.1128/jcm.28.9.1903-1905.1990>
- Hunter, P. R., & Gaston, M. A. (1988). Numerical index of the discriminatory ability of typing systems: An application of Simpson's index of diversity. *Journal of Clinical Microbiology*, 26(11), 2465-2466. <http://jcm.asm.org/content/26/11/2465.abstract>
- Inokuti, E. M., Reis, A., Ceresini, P. C., Câmara, M. P. S., & Michereff, S. J. (2019). Diversity and pathogenicity of anastomosis groups of *Rhizoctonia* associated with potato stem canker and black scurf diseases in Brazil. *European Journal of Plant Pathology*, 153(4), 1333-1339. <https://dx.doi.org/10.1007/s10658-018-01627-5>
- Jia, Y., Correa-Victoria, F., McClung, A., Zhu, L., Liu, G., Wamishe, Y., & Correll, J. C. (2007). Rapid determination of rice cultivar responses to the sheath blight pathogen *Rhizoctonia solani* using a micro-chamber screening method. *Plant Disease*, 91(5), 485-489. <https://dx.doi.org/10.1094/pdis-91-5-0485>
- Justesen, A. F., Yohalem, D., Bay, A., & Nicolaisen, M. (2003). Genetic diversity in potato field populations of *Thanatephorus cucumeris* AG-3, revealed by ITS polymorphism and RAPD markers. *Mycological Research*, 107(11), 1323-1331. <https://dx.doi.org/10.1017/S0953756203008517>
- Kuninaga, S., Carling, D. E., Takenuichi, T., & Yokosawa, R. (2000). Comparison of rDNA-ITS Sequences between Potato and Tobacco Strains in *Rhizoctonia solani* AG-3. *Journal of General Plant Pathology*, 66(1), 2-11. <https://dx.doi.org/10.1007/PL00012917>
- Kuninaga, S., Godoy-Lutz, G., & Yokosawa, R. (2002). rDNA-ITS nucleotide sequences analysis of *Thanatephorus cucumeris* AG-1 associated with web blight on common beans in Central America and Caribbean. *Japanese Journal of Phytopathology*, 68, 3-20.
- Lees, A. K., Cullen, D. W., Sullivan, L., & Nicolson, M. J. (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 Pathology*, 51(3), 293-302. <https://dx.doi.org/10.1046/j.1365-3059.2002.00712.x>
- Mandujano, M. C., Montaña, C., Franco, M., Golubov, J., & Flores-Martínez, A. (2001). Integration of demographic annual variability in a clonal desert cactus. *Ecology*, 82(2), 344-359. [https://dx.doi.org/10.1890/0012-9658\(2001\)082\[0344:IODAVI\]2.0.CO;2](https://dx.doi.org/10.1890/0012-9658(2001)082[0344:IODAVI]2.0.CO;2)

- Mandujano, M. del C., Montaña, C., Méndez, I., & Golubov, J. (1998). The relative contributions of sexual reproduction and clonal propagation in *Opuntia rastrera* from two habitats in the Chihuahuan Desert. *Journal of Ecology*, 86(6), 911-921. <https://dx.doi.org/10.1046/j.1365-2745.1998.00308.x>
- McDonald, B. A. (1997). The population genetics of fungi: tools and techniques. *Phytopathology*, 87(4), 448-453. <https://dx.doi.org/10.1094/PHYTO.1997.87.4.448>
- McDonald, B. A., & Linde, C. (2002). Pathogen population genetics, evolutionary potential, and durable resistance. *Annual Review of Phytopathology*, 40(1), 349-379. <https://dx.doi.org/10.1146/annurev.phyto.40.120501.101443>
- Mogie, M., & Hutchings, M. (1990). Phylogeny, ontogeny and clonal growth in vascular plants. In J. van Groenendael & H. de Kroon (Eds.), *Clonal Growth in Plants: Regulation and Function* (pp. 3-22). SPB Academic Publishing.
- Muzhinji, N., Truter, M., Woodhall, J. W., & Van der Waals, J. E. (2015). Anastomosis groups and pathogenicity of *Rhizoctonia solani* and binucleate *Rhizoctonia* from potato in South Africa. *Plant Disease*, 99(12), 1790-1802. <https://dx.doi.org/10.1094/PDIS-02-15-0236-RE>
- Muzhinji, N., Woodhall, J. W., Truter, M., & Van der Waals, J. E. (2017). Relative contribution of seed tuber- and soilborne inoculum to potato disease development and changes in the population genetic structure of *Rhizoctonia solani* AG 3-PT under Field Conditions in South Africa. *Plant Disease*, 102(1), 60-66. <https://dx.doi.org/10.1094/pdis-03-17-0329-re>
- Nei, M. (1973). Analysis of Gene Diversity in Subdivided Populations. *Proceedings of the National Academy of Sciences*, 70(12), 3321-3323. <https://doi.org/10.1073/pnas.70.12.3321>
- Ogoshi, A. (1987). Ecology and Pathogenicity of Anastomosis and Intraspecific Groups of *Rhizoctonia Solani* Kuhn. *Annual Review of Phytopathology*, 25(1), 125-143. <https://dx.doi.org/10.1146/annurev.py.25.090187.001013>
- Pereira, D. A., Ceresini, P. C., Castroagudín, V. L., Ramos-Molina, L. M., Chavarro-Mesa, E., Negrisola, M. M., & Takada, H. M. (2016). Population genetic structure of *Rhizoctonia oryzae-sativae* from rice in Latin America and its adaptive potential to emerge as a pathogen on *Urochloa* pastures. *Phytopathology*, 107(1), 121-131. <https://dx.doi.org/10.1094/phyto-05-16-0219-r>
- Pérez, T., Albornoz, J., & Dominguez, A. (1998). An evaluation of RAPD fragment reproducibility and nature. *Molecular Ecology*, 7(10), 1347-1357. <https://dx.doi.org/10.1046/j.1365-294x.1998.00484.x>
- Poloni, N. M., Molina, L. M. R., Mesa, E. C., Garcia, I. L., & Ceresini, P. C. (2016). Evidência de que o fungo *Rhizoctonia solani* AG-1 IA adaptado à *Urochloa* na Colômbia mantém ampla gama de hospedeiros incluindo o milho. *Summa Phytopathologica*, 42(3), 228-232. <https://dx.doi.org/10.1590/0100-5405/2176>
- R Core Team. (2017). R: *A language and environment for statistical computing* [software]. R Foundation for Statistical Computing. <https://www.r-project.org/>
- Ramanatha, V., & Hodgkin, T. (2002). Genetic diversity and conservation and utilization of plant genetic resources. *Plant Cell, Tissue and Organ Culture*, 68(1), 1-19. <https://dx.doi.org/10.1023/A:1013359015812>
- Ramos-Molina, L. M., Chavarro-Mesa, E., Pereira, D. A. dos S., Silva-Herrera, M. del R., & Ceresini, P. C. (2016). *Rhizoctonia solani* AG-1 IA infects both rice and signalgrass in the Colombian Llanos. *Pesquisa Agropecuária Tropical*, 46(1), 65-71. <https://dx.doi.org/10.1590/1983-40632016v4638696>
- Rioux, R., Manmathan, H., Singh, P., De Los Reyes, B., Jia, Y., & Tavantzis, S. (2011). Comparative analysis of putative pathogenesis-related gene expression in two *Rhizoctonia solani* pathosystems. *Current Genetics*, 57(6), 391-408. <https://dx.doi.org/10.1007/s00294-011-0353-3>

- Roberts, P. (2000). *Rhizoctonia*-Forming Fungi, a Taxonomic Guide. *Kew Bulletin*, 55(1), 252-253. <https://dx.doi.org/10.2307/4117793>
- Salazar-Zuluaga, L. (2014). *Creation of descriptive diagrams for the severity of "take-all" disease by Gaeumannomyces graminis Sacc. Von Arx & d. Oliver var. graminis in leaves and stalks through different phenological stages of rice*. [Master's thesis, Universidad Nacional de Colombia]. Repositorio UN. <https://repositorio.unal.edu.co/handle/unal/75277>
- 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(6), 299-316. <https://dx.doi.org/10.1007/s10267-006-0320-x>
- Suzuki, R., & Shimodaira, H. (2006). Pvcust: An R package for assessing the uncertainty in hierarchical clustering. *Bioinformatics*, 22(12), 1540-1542. <https://dx.doi.org/10.1093/bioinformatics/btl117>
- Tsrör, L. (2010). Biology, epidemiology and management of *Rhizoctonia solani* on potato. *Journal of Phytopathology*, 158(10), 649-658. <https://dx.doi.org/10.1111/j.1439-0434.2010.01671.x>
- Willi, Y., Frank, A., Heinzemann, R., Kälin, A., Spalinger, L., & Ceresini, P. C. (2011). The adaptive potential of a plant pathogenic fungus, *Rhizoctonia solani* AG-3, under heat and fungicide stress. *Genetica*, 139(7), 903-908. <https://dx.doi.org/10.1007/s10709-011-9594-9>
- Woodhall, J. W., Adams, I. P., Peters, J. C., Harper, G., & Boonham, N. (2013). A new quantitative real-time PCR assay for *Rhizoctonia solani* AG3-PT and the detection of AGs of *Rhizoctonia solani* associated with potato in soil and tuber samples in Great Britain. *European Journal of Plant Pathology*, 136(2), 273-280. <https://dx.doi.org/10.1007/s10658-012-0161-8>
- Yang, S., Min, F., Wang, W., Wei, Q., Guo, M., Gao, Y., Lu, D. (2017). Anastomosis group and pathogenicity of *Rhizoctonia solani* associated with stem scanker and black scurf of potato in Heilongjiang Province of China. *American Journal of Potato Research*, 94(2), 95-104. <https://dx.doi.org/10.1007/s12230-016-9535-3>
- Yang, Y., Zhao, C., Guo, Z., & Wu, X. (2015). Anastomosis group and pathogenicity of *Rhizoctonia solani* associated with stem canker and black scurf of potato in China. *European Journal of Plant Pathology*, 143(1), 99-111. <https://dx.doi.org/10.1007/s10658-015-0668-x>