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Morphology and pathogenicity of *Rhizoctonia solani* Kühn associated with potato black scurf in Nariño (Colombia)

Morfología y patogenicidad de *Rhizoctonia solani* Kühn asociado con la costra negra de la papa en Nariño (Colombia)

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***Solanum tubersoum* var. Red tubers with black scurf caused by *Rhizoctonia solani*.**

Photo: B.L. Castro

ABSTRACT

The objective of the research was to understand the diversity of *Rhizoctonia solani* in potato crops in Nariño. Tubers with sclerotia were collected from farms in the municipalities of Pasto, Ipiales, Tuquerres and Ospina. In a laboratory, the strains were grouped in categories, selecting 30 for morphological and pathogenic studies. In an PDA medium, the daily mycelia growth rate (DMGR), pigmentation, texture, growth pattern (GP) and sclerotia characteristics were determined. The hyphae width and nuclei number were also evaluated. *Solanum tuberosum* L. Group Phureja seedlings were used in the pathogenicity test. Initially, 494 strains were obtained with diverse cultural characteristics, grouped in 15 categories, selecting two of each one for the research. Of the 30 strains, there were significant differences in the DMGR according to the Tukey test ($P=0.05$), 96.6% of the strains had an average of 16.6 mm day^{-1}. 15 day-old colonies had cream, beige, brown and salmon colors. 95% of the isolates formed plush mycelium with GP concentric simple rings, complex rings, and scattered and stellate forms. Sclerotia formation began at 6 days (average), and, at 15 days, dispersed arrangement predominated, as well as a peripheral, with brown, beige and cream colors. Three isolates did not produce sclerotia. The hyphae had a mean of 9.7 μm, and the nuclei number ranged between 7.2 - 8.2, without statistical differences. Twenty-four isolates caused 100% plant infection. The results suggest differences between the isolates, associated with levels of pathogenicity or anastomosis groups (AG), characteristics that will be studied in future research.

Additional key words: stem canker; soil-borne fungi; sclerotia; *Solanum tuberosum.*

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RESUMEN

El objetivo de la investigación fue conocer la diversidad de *Rhizoctonia solani* en cultivos de papa en el departamento de Nariño. Se colectaron tubérculos con esclerocios en fincas de los municipios de Pasto, Ipiales, Túquerres y Ospina. En laboratorio, los aislamientos se agruparon en categorías, seleccionando 30 para los estudios morfológicos y patogénicos. En medio PDA, se determinó la tasa diaria de crecimiento micelial (TDCM), color, textura, patrón de crecimiento (PC) y características de los esclerocios. Se evaluó ancho de hifas y número de núcleos. Para pruebas de patogenicidad se utilizaron plántulas de papa *Solanum tuberosum* L. Group Phureja. Se obtuvieron 494 aislamientos con características culturales variadas, agrupados en 15 categorías, seleccionando dos de cada grupo para la investigación. Los 30 aislamientos mostraron diferencias significativas en la TDCM y según prueba de Tukey (P=0,05%), el 96,6% tuvo un promedio de 16,6 mm día^{-1}. Colonias de 15 días de edad presentaron colores crema, beige, marrón y salmón. El 95% de los aislamientos formaron micelio afelpado con PC radial simple, radial complejo, disperso y estrellado. La formación de esclerocios se inició a los 6 días y a los 15 mostraron disposición dispersa y periférica, con colores marrón, beige y crema. Tres aislamientos no produjeron esclerocios. El ancho de hifas tuvo un promedio de 9,7 μm y entre 7.2-8.2 núcleos, sin diferencias estadísticas. Veinticuatro aislados causaron 100% de infección en las plantas. Los resultados sugieren diferencias entre aislamientos, asociados con patogenicidad o grupos de anastomosis, características que serán estudiadas en futuras investigaciones.

Palabras clave adicionales: cancro de tallo; hongos del suelo; esclerocios; *Solanum tuberosum.*

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INTRODUCTION

In the Nariño Department, potato production ranks third in Colombia, and 24,906 ha were planted in 2019, with a yield average of approximately 578,695 t (FEDEPAPA, 2019). The many phytosanitary problems that affect potato cultivation include the black scurf and stem canker caused by the fungus *Rhizoctonia solani* Kühn (Tsror, 2010; ICA, 2011; Álvarez-Sánchez *et al*., 2018). *Rhizoctonia solani* causes damage in all stages of potato development crops, including emerging shoots, young neck tissues of the seedlings, roots and stolons, where necrotic brown lesions can strangle them. In adult plants, necrotic lesions interfere with the normal movement of nutrients, inducing the formation of aerial tubers in the axils of the leaves. Developing tubers remain small or deformed, and the surface of tubers have black crusts or sclerotia, detracting from quality (Tsror, 2010; ICA, 2011). Worldwide, this disease causes important decreases in yield and quality and is responsible for losses of up to US$ 75million/year in several countries (Das *et al*., 2014).

The fungus *R. solani* (anamorph of *Thanatephorus cucumeris* (Frank.) Donk) belongs to Class Basidiomycetes. The hyphae are dark brown, septate and multinucleated, where cells have 2-18 nuclei (Misawa and Kurose, 2018, 2019). The hyphae fusion, known as anastomosis, is a particular characteristic of this species (Carling, 1996). This condition has been considered for classification in anastomosis groups (AGs), which differ from each other in phylogenetic, morphology, physiology and pathogenicity (Fiers *et al*., 2011; Ferrucho *et al*., 2012; Muzhinji *et al*., 2015). To date, 13 AGs has been identified worldwide, and the more important are AG3 and AG4. AG3 is the most pathogenic and is characterized mainly by the formation of sclerotia on tubers (Ferrucho *et al*., 2012; Das *et al*., 2014; Muzhinji *et al*., 2015). In Venezuela, Ecuador and Colombia, AG3 has been determined as the most pathogenic in potato cultivars (Escalona *et al*., 2011; Alban, 2015; Ferrucho *et al*., 2012, 2013).

The study of population genetics of plant pathogens is useful for understanding epidemiology, ecology and evolutionary trajectory to effectively estimate the pathogen potential evolution and dispersion under natural ecosystems. This knowledge evaluates the sensitivity of pathogen isolates for agrochemicals and development of resistant cultivars (Zhan, 2016; Chañag *et al*., 2018). Because of the importance of *R. solani* in potato production in the Nariño Department, the objective of this research was to obtain a

preliminary identification and characterization of *R. solani* populations in the region. The following studies expand knowledge of this pathogen and evaluate management strategies.

MATERIALS AND METHODS

Sampling and isolation of *R. solani*

The sample collection was done from September to November, 2019, on commercial potato farms in the municipalities of Pasto, Ipiales, Tuquerres and Ospina, south of Colombia (1°12' N and 77°16 W to 0°49 N and 77°38' W), at altitudes of 2,915 to 3,179 m a.s.l. The laboratory work was carried out in the phytopathology laboratory of the Faculty of Agricultural Sciences of the Universidad de Nariño (Pasto, Colombia), with an altitude of 2,527 m a.s.l. In each municipality, potato crops in the harvest stage were randomly selected, collecting 10 tubers with sclerotia/place. The sclerotia were removed using a scalpel, disinfested with sodium hypochlorite (3%), placed on potato dextrose agar (PDA) containing streptomycin sulphate (20 mg L^{-1}), and incubated for 3 d at 25°C, according Das *et al*. (2014) and Misawa *et al*. (2018). *Rhizoctonia*-like fungi colonies were identified according to Sneh *et al*. (1991), and the purification was done with the hyphal tipping technique on PDA.

Morphological characterization of isolates

The strains were grouped according cultural appearance, such as pigmentation, growth pattern (GP), and pattern of sclerotia formation (PSF), according Escalona *et al*. (2011) and Dubey *et al.* (2014). Of these groups, 30 strains were selected to continue the morphological study on PDA and incubation in dark for 15 d at 25°C. Four repetitions were made for each isolate, placing 3 mm diameter plugs taken from the colony margins in the center of the plates. Strains were monitored daily for the following characteristics, according Dubey *et al*. (2014): - Mycelial growth, measuring the colony diameter (mm/ day), mean of two measurements; pigmentation, with evaluation at 8 and 15 d, according to a color chart (Kramer, 2004); - mycelium texture: cottony (aerial mycelium), plush attached to the medium or grainy; - mycelial growth pattern (MGP) at 15 d, classified according to Dubey *et al*. (2014) as: central, simple radial, complex radial, dispersed and star-shaped; - Sclerotia characteristics: PSF, as peripheral, central, scattered, star-shaped or absent; size (using the software of imageJ program), categorized as microsclerotia (<1 mm) and macrosclerotia (>1 mm); shape (regular, irregular, globular and powdery); coloration and amount visually estimated. According to the sclerotia abundance, the isolates were classified as: 1-100 (scarce), 100-200 (moderate) and > 200 (abundant), as reported by Dubey *et al*. (2014).

The mycelial growth rate (MGR) was obtained in mm day^{-1} using the method of Dubey *et al*. (2014), and the data were subjected to ANAVA and Tukey's test (P = 0.05%). According to the MGR, the isolates were classified as: slow (10 mm day^{-1}), medium (> 10-12 mm day^{-1}) and fast (> 12 mm day^{-1}). With qualitative variables (pigmentation, mycelium texture, MGP and PSF), the averages of the proportion (%) of isolates were obtained for each one.

Microscopic characteristics were made using method of Gondal *et al*. (2019); slides with mycelium printed with adhesive tape and stained with lactophenol blue (0.05%) were used to observe the hyphal morphology of *R. solani,* (four plates/repetition) in 8-d-old cultures. The morphology of each culture was compared with previous descriptions, including width and length of the hyphal (Misawa *et al*., 2018). Number of nuclei per cell were counted by staining the hyphae with Safranin O + a drop of 3% KOH on a portion of mycelium 48 h after incubation (Bandoni, 1979). Five observations of each characteristic/repetition were examined microscopically at 100X. The data were subjected to ANOVA and Tukey's test (P=0.05).

Pathogenicity tests

The inoculum was prepared by colonizing isolates of *R. solani* on rice grains for 18 d (Castro *et al*., 2013). Potato seedlings (*S. tuberosum* Group Phureja) sown in plastic cups (one tuber/cup) were used. The seed-tubers were checked to be free of sclerotia and disinfested in water with 1% sodium hypochlorite. Each cup contained 350 g of a soil-rice husk mixture (3:1), sterilized with hot water. Eighteen-day-old seedlings were inoculated with 6.0 g of rice colonized with each isolate; the inoculum was mixed in the upper 4 cm layer of the soil. Five plants were inoculated by strain, and a control free of pathogen was included. The plants were placed on tables under a mesh house, at 11-20°C. Thirty-five days after inoculation, plant infection was evaluated, observing secondary

symptoms (wilting and yellowing): lesions on roots, shoots, stolons or stem canker (primary symptoms). The results were expressed as a percentage of plants infected by isolate. Re-isolations of the pathogen were done to confirm the pathogenic nature of the isolates.

RESULTS AND DISCUSSION

Sampling and isolation of *R. solani*

The pathogen was 100% prevalent in all locations, where tubers with sclerotia were found, indicating the presence of this pathogen in the potato production area of the department. Out of the 50 samples, 17 were found in crops in Pasto, 12 in Ipiales, 9 in Tuquerres and 12 in Ospina. The commercial potato varieties with *R. solani* included the species *S. tuberosum* and *S. tuberosum* Group Phureja (Fig. 1). The sclerotia observed on the tubers had dark brown and black colors, with varied shape and size, from 1 to 4 mm in diameter, as reported by Misawa and Kurose (2018). From the sclerotia, 494 pure isolates were obtained with typical features of *R. solani:* hyphae with right-angle branches at the distal septae of cells, dolipore septum, and constriction at the branch, similar characteristics described by Sneh *et al.* (1991). No conidia or conidiophores were observed.

In this study, the pathogen sampling focused exclusively on the collection of tubers with sclerotia (Atkinson *et al*., 2010; Dubey *et al*., 2014; Muzhinji *et al*., 2015), or even from soil (Alban, 2015). The *R. solani* isolation from the sclerotia of commercial varieties of *S. tuberosum,* as reported by Escalona *et al*. (2011) for Venezuela, by Ferrucho *et al*. (2012) in Colombia, and, in Ecuador, by Alban (2015), obtained isolates from soil.

Morphological characterization of isolates

The 494 isolates showed diversity in pigmentation, GP and PSF at 15 d after incubation, as reported by Misawa and Kurose (2018). Predominantly white colonies and cream, light brown and salmon colors, with simple radial, complex radial and star-shapes were noticed. The sclerotia were arranged in the center, periphery, scattered or absent. These diversities

Figure 1. Potato tubers with *R. solani* sclerotia. A) *Solanum tuberosum* var. Unique, B) *S. tuberosum* var. Parda Suprema, C) *S. tuberosum* Group Phureja, D) *S. tuberosum* var. Diacol Capiro, E) *S. tuberosum* var. Superior and F) *S. tuberosum* var. Red Huila.

coincide with several authors (Escalona *et al*., 2011; Oliveira *et al*., 2014; Abdel-Sattar *et al*., 2017), who suggested that such variability may be related to the culture medium, temperature, colony age, AG group or host plant species.

Based on the cultural features expressed by the *R. solani* isolates, 15 groups were classified, as shown in table 1. A variable number of isolates of each group were selected, with 30 isolates for the morphology and pathogenicity (Tab. 2).

Table 1. Isolates of *R. solani* grouped according cultural features in the PDA medium.

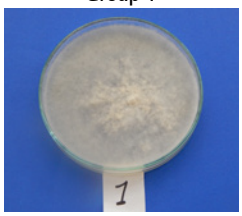
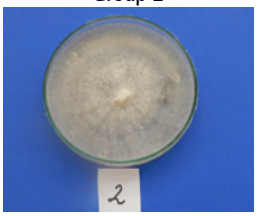
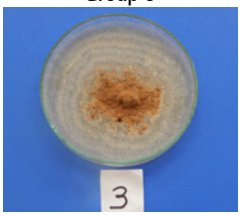
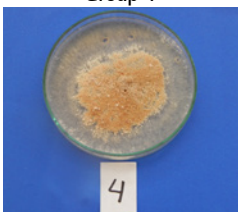
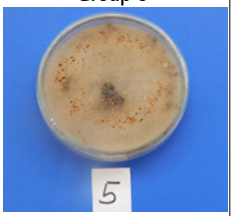
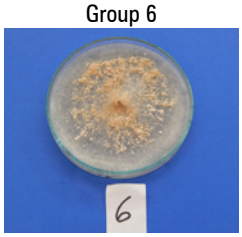
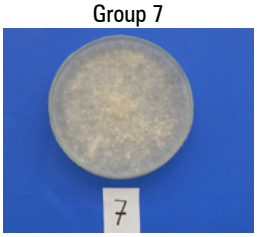
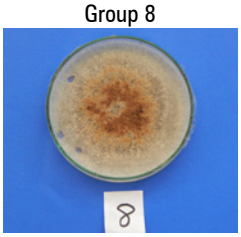
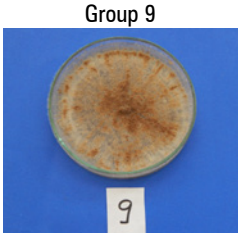
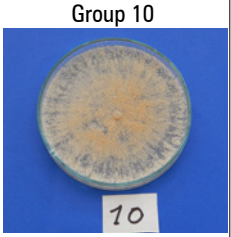
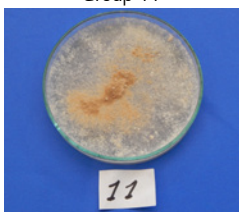
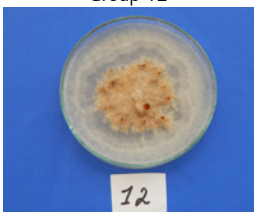
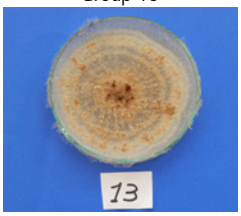
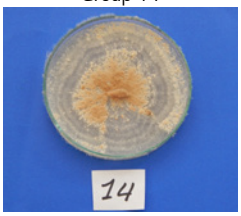
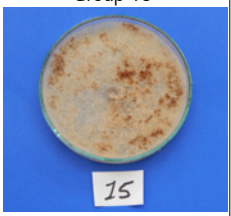
Group 1  Plush and white mycelium attached to the medium. No sclerotia	Group 2  Plush and white mycelium attached to the medium. Late and white sclerotia formation	Group 3  Plush and cream mycelium attached to the medium, with radial complex circles. Central and brown sclerotia	Group 4  Plush and salmon mycelium, attached to the medium, with simple radial growth central and salmon sclerotia	Group 5  Abundant cottony mycelium, aerial, brown, with complex radial growth. Brown sclerotia formation on the lid
Group 6  Pale salmon colony, mycelium attached to the medium. Scattered salmon sclerotia	Group 7  Pale cream colony. Abundant aerial mycelium. Scattered white sclerotia	Group 8  Mycelium and sclerotia brown, mostly central or scattered	Group 9  White colony. Brown sclerotia in radial growth, towards the edge	Group 10  Pale salmon colony, star-shaped growth. Scattered and abundant salmon sclerotia
Group 11  Dark salmon colony, sparse mycelium. Sclerotia scattered, dark brown or light brown	Group 12  Pale cream colony, with radial growth and wavy edges. Cream sclerotia scattered with gum exudates	Group 13  Cream and fluffy mycelium, with wavy edges. Beige, scattered and central sclerotia	Group 14  Sparse and cream mycelium, granular (pasty) texture and wavy edges. Central brown sclerotia	Group 15  Sparse dark cream and plush mycelium. Scattered brown sclerotia

Table 2. *Rizoctonia solani* isolates and cultural characteristics identified in this study.

Isolate *	Location of collection	Potato variety		Mycelial growth rate (mm/day) ***	Nuclei number ***	Hyphal width-length μm ***
RsPN-400	Pasto	*S. tub.* G. Phureja (Criolla amarilla)	3	18.13±0.09 a	7.3±0.3 a	10.1±1.0 a 108.9±13.9 a

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Table 2. ***Rizoctonia solani*** **isolates and cultural characteristics identified in this study.**

Isolate *	Location of collection	Potato variety	Group **	Mycelial growth rate (mm/day) ***	Nuclei number	Hyphal width-length μm
RsPN-232	Pasto	*S. tub.* G. Phureja (Puerreña)	13	16.87±0.16 abcde	6.3±0.4	8.5±0.7 a 99.9±13.6
RsPN-135	Pasto	*S. tuberosum* (Parda Suprema)	9	16.84±0.27 abcde	5.7±0.3	8.8±0.5 a 82.9±6.3
RsPN-183	Pasto	*S. tuberosum* (Diacol Capiro)	13	16.80±0.27 abcde	5.3±0.6	9.9±0.3 a 93.4±5.0
RsPN-109	Pasto	*S. tuberosum* (Única)	1	16.65±0.20 abcde	6.7±0.8	8.8±0.5 a 105.8±9.4
RsPN-195	Pasto	*S. tuberosum* (Diacol Capiro)	1	16.60±0.09 abcde	6.6±0.4	9.5±0.9 a 90.4±12.0
RsPN-325	Pasto	*S. tuberosum* (Superior)	2	16.58±0.11 abcde	5.8±0.1	8.1±0.7 a 72.7±2.5
RsPN-158	Pasto	*S. tuberosum* (Diacol Capiro)	11	15.99±0.35 bcde	6.5±0.4	10.5±1.3 a 102.9±3.2
RsPN-175	Pasto	*S. tuberosum* (Diacol Capiro)	8	14.98±0.96 e	6.4±0.5	8.1±0.1 a 118.4±18.9
RsPN-24	Ipiales	*S. tuberosum* (Diacol Capiro)	5	16.94±0.22 abcde	7.2±1.0	7.9±0.2 a 74.1±1.7
RsPN-7	Ipiales	*S. tub.* G. Phureja (SuaPa)	11	16.88±0.13 abcde	8.2±0.8	8.3±0.6 a 94.3±8.9
RsPN-346	Ipiales	*S. tub.* G Phureja (Ocarina)	15	16.76±0.15 abcde	6.3±0.6	8.1±0.6 a 106.3±11.5
RsPN-345	Ipiales	*S. tuberosum* (Diacol Capiro)	15	16.53±0.42 abcde	7.0±0.4	8.2±0.2 a 96.4±1.4
RsPN-374	Ipiales	*S. tuberosum* (Diacol Capiro)	3	16.46±0.26 abcde	8.0±1.3	7.4±0.2 a 104.9±14.1
RsPN-3	Ipiales	*S. tuberosum* (Superior)	10	16.28±0.25 abcde	6.1±0.2	11.2±0.8a 88.7±12.9
RsPN-36	Tuquerres	*S. tuberosum* (Roja Huila)	6	17.66±0.20 abc	6.4±0.3	7.4±0.1 a 114.2±12.1
RsPN-274	Tuquerres	*S. tuberosum* (Diacol Capiro)	6	18.09±0.16 a	5.8±0.4	7.8±0.4 a 75.0±3.7
RsPN-269	Tuquerres	*S. tuberosum* (Diacol Capiro)	8	17.07±0.15 abcd	6.3±0.7	9.2±0.8 a 92.6±6.3
RsPN-262	Tuquerres	*S. tuberosum* (Diacol Capiro)	5	16.98±0.11 abcd	4.9±0.2	7.9±0.3 a 101.6±2.3
RsPN-290	Tuquerres	*S. tuberosum* (Diacol Capiro)	4	16.94±0.02 abcde	6.2±0.8	12.9±4.9 a 91.0±19.6
RsPN-99	Tuquerres	S. *tuberosum* (Única)	12	15.79±0.23 cde	5.9±0.3	7.8±0.5 a 84.1±5.7
RsPN-289	Tuquerres	*S. tuberosum* (Diacol Capiro)	12	12.43±1.34 f	5.2±0.1	8.7±0.5 a 99.2±5.2
RsPN-487	Ospina	*S. tuberosum* (Única)	4	17.85±0.09 ab	6.3±0.5	8.6±0.1 a 95.9±5.0

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Table 2. ***Rizoctonia solani*** **isolates and cultural characteristics identified in this study.**

Isolate *	Location of collection	Potato variety	Group **	Mycelial growth rate (mm/day) ***	Nuclei number	Hyphal width-length μm
RsPN-469	Ospina	*S. tuberosum* (Diacol Capiro)	9	16.87±0.14 abcde	5.8±1.0	8.0±0.6 a 102.5±6.8
RsPN-434	Ospina	*S. tuberosum* (Diacol Capiro)	14	17.31±0.14 abc	7.3±0.3	8.1±0.5 a 77.6±3.4
RsPN-484	Ospina	*S. tuberosum* (Diacol Capiro)	14	17.30±0.17 abc	5.8±0.5	8.4±0.3 a 90.5±3.8
RsPN-432	Ospina	*S. tuberosum* (Diacol Capiro)	10	17.21±0.17 abcd	6.9±0.5	8.6±0.3 a 86.0±4.3
RsPN-482	Ospina	*S. tuberosum* (Diacol Capiro)	2	16.45±0.14 abcde	6.9±0.8	6.6±0.1 a 103.2±3.5
RsPN-486	Ospina	*S. tuberosum* (Única)	7	16.34±0.09 abcde	6.7±0.6	10.0±0.8 a 89.2±2.4
RsPN-442	Ospina	*S. tuberosum* (Diacol Capiro)	7	15.28±0.15 de	6.5±0.7	8.4±0.8 a 106.5±8.1

*Isolates encoded: *R. solani* (Rs), potato (P), Nariño (N).

** Groups according cultural preliminary features on PDA (related to Tab. 1).

***Values are mean±standard error. Means followed by the same letter in a columns do not differ according to Tukey's test.

Strains pigmentation. During the first 2 d, the colonies formed hyaline mycelium that adhered to the medium, characteristic of *R. solani* populations according to Dubey *et al*. (2014). In the following days, the mycelium had different pigmentations: white, cream to pale beige. According to the color table (Kramer, 2004), at 8 d-old, pale brown predominated in 50% of the isolates, with cream in 30%, and the rest had the two colors. At 15 d, pale brown was the predominant color in 26% of the isolates, with cream in 10%, beige in 10% and brown in 6.6% of the isolates. The rest of colonies had similar colors, two were salmon. Several authors (Muzhinji *et al*., 2015; Abdel-Sattar *et al*., 2017) have reported that the pigmentation of *R. solani* colonies is a variable characteristic exhibited by isolates from all species of plants affected by this pathogen. It appears to be related to the culture medium, temperature, age, and possibly the genetics of the fungus. In potato isolates, Misawa and Kurose (2018) found white pale brown, slightly gray and brown colors; while Muzhinji *et al*. (2015) reported *R. solani* isolates in different AG groups, with white to brown mycelium. Oliveira *et al*. (2014) found colonies with colors from beige to brown. In the present study, this characteristic was probably associated with strain age in the PDA medium.

Strain texture. At 8 d-old, the plush mycelium adhered to the medium in 63.3% of isolates, as reported by Carling and Leiner (1990). Cottony and raised mycelium was observed in 25% of the isolates, and the rest had lower proportions of both types. Four isolates had granular texture. After 15 d, 94% of the isolates had plush mycelium adhered to the medium, and the rest of colonies exhibited cottony, sparse, aerial mycelium adhered to the lid of the Petri dish. This last characteristic was not highly variable although several authors have reported colonies with abundant aerial mycelium (Abdel-Sattar *et al.,* 2017; Misawa and Kurose, 2019).

Strain growth pattern. The varied GP initially exhibited colonies, and it changed over time, as reported by Abdel-Sattar *et al*. (2017). A simple radial pattern had 30% of isolates at 8 d-old, followed by 13.5% with a complex radial pattern (several circles). The rest of the isolates had varied forms, such as scattered and star-shaped. At 15 d, 26.6% of the isolates had complex radial forms, followed by 23.3% with a simple radial, and the rest exhibited scattered and star forms (Fig. 2). This variability, as reported by Abdel-Sattar *et al*. (2017) and Misawa and Kurose (2019), coincided in some cases with the formation of simple and multiple concentric circles, changing over time.

Sclerotia characteristics. Sclerotia formation began at 6 d-old in 86% of the strains, similar to that described by Abdel-Sattar *et al*. (2017) and Gondal *et*

al. (2019), with varying colorations between white, cream and brown, either rough or smooth. Fifteen days later, 35% of the isolates showed brown sclerotia, followed by beige (25%) and cream (15%). The rest were white, cream and orange. Three isolates did not form sclerotia (Fig. 2A). The 27 strains showed varied PSF; 50% were scattered, 40% were peripheral, and the rest were central, with a star shape, or combined both star/peripheral (Fig. 2A), similar to the results of Misawa and Kurose (2019). Forty-six percent of the strains were classified with a low sclerotia/plate (1-100), 42% were moderate (between > 100-200), and 11.5% had abundant sclerotia (> 200) (Fig. 2B). The size and shape of the sclerotia was variable; macro (>1 mm) and microsclerotia (<1 mm), jointly in 90% of the isolates, with irregular or undefined shapes, and the rest presented microsclerotia with regular borders (Fig. 2C). These results coincide with those recorded by Dubey *et al*. (2014), who reported sclerotia diameters between 0.10-5.4 mm and with a peripheral, central and dispersed arrangement. Abdel-Sattar *et al*. (2017) reported the formation of brown sclerotia, 5.0 mm in diameter, while Escalona *et al*. (2011) found abundant dark brown sclerotia located only in the center of the colonies, similar to Misawa and Kurose (2019).

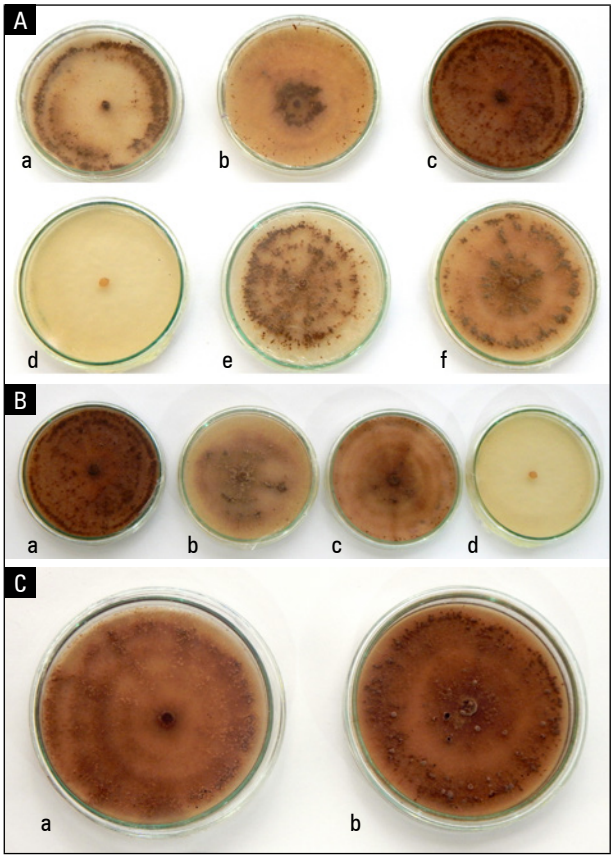

Figure 2. Mycelial growth pattern and sclerotia of *R. solani* on PDA. A) Arrangement, a: peripheral, b: central, c: scattered, d: absent, e: starry and f: starry/peripheral. B) Quantity, a: abundant, b: moderate, c: scarce, d: absent. C) Size, a: microsclerotia, b: macrosclerotia and microsclerotia.

Mycelial growth rate. This variable was evaluated during the first 6 d, when all isolates filled the plate. All strains had rapid growth, as observed by Dubey *et al*. (2014), with a MGR higher of 12 mm d^{-1}. Although the ANOVA showed statistical differences ($P<0.0001$) for the rate of mycelial growth between the isolates, no relationship was observed with the 15 initially determined morphological groups, neither with the geographic location of the cultures nor with the potato variety. According to Tukey's test ($P=0.05$), only one isolate (RsPN-289) had the lowest MGR with 12.43 mm d^{-1}, and the maximum was 18.13 mm d^{-1} (RsPN-400) (Tab. 2). Escalona *et al*. (2011) and Dubey *et al*. (2014) indicated that the MGR of *R. solani* depends on the culture medium and the incubation temperature. At 10°C, the full plate can occur between 7-11 d, while, at 20°C, it occurs in 4 d. At 30°C, it occurs in 16 d. In our study, the full of plate was in 6 d at 25°C. However, the MGR may be related to AG groups or pathogenicity level, aspects that will be addressed to the next phase of the research.

Microscopic characterization. All isolates showed the typical characteristics of *R. solani*: hyphae with

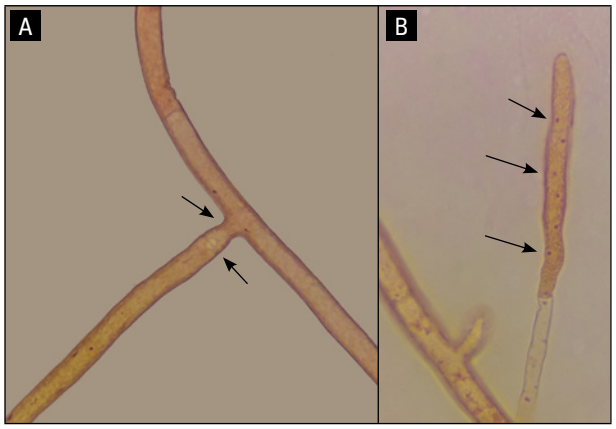

Figure 3. Typical features of *R. solani* under light microscope (100X). A) septate hypha with right-angle branch, constriction at the point of branching and septum close to the origin. B) Formation of multiple nuclei.

right-angle branches, branches at the distal septae of cells, dolipore septa, constriction at the junction of these hyphae, and multinucleate cells (Fig. 3 A and B), as described by Dubey *et al*. (2014) and Abdel-Sattar *et al.* (2017). No statistical differences were found in hyphae dimensions; the width ranged between 6.6 and 12.9 μm (average 8.7 μm), and the hyphal distance between two septae varied from 72.7 to 118.4 μm (average 95.0 μm) (Tab. 2). These values are similar to those reported by Dubey *et al.* (2014) —4.1-10.3 μm—, who found some isolates with values of > 8 in the groups AG1 to AG5. No differences were found in nuclei number, with a minimum of 4.9 and maximum of 8.2, within the ranges reported by Tsror (2010) and Misawa and Kurose (2019) with (4.4-10.9 nuclei).

Pathogenicity tests

Thirty-five days after inoculation, some plants showed secondary symptoms, such as yellowing, wilting and reduction of plant development, as compared to the control (Fig 4A). Reduction of root area, necrotic lesions in root tips and sections, in stolons

Figure 4. Symptoms caused by different strains of *R. solani* in potato seedlings (*S. tuberosum* Group Phureja). A-B) Secondary symptoms and reduction of roots, as compared to the control (far left), and C) Necrotic lesions on roots.

and in some cases in shoot tissues were observed (Fig. 4 B and C). Out of 30 isolates, 24 caused root lesions in 100% of the plants, as well as reduction of more than 50% of roots, as compared with the control, coinciding with symptoms mentioned by several authors (Abdel-Sattar *et al*., 2017). These 24 isolates produced some small sclerotia on developing tubers (12.5-100% of plants). Six isolates caused both secondary and primary symptoms (50- 87.5% of plants). No symptoms of stem canker were observed. External symptoms, mainly plant reduction of development, were related with primary symptoms at the variable level. Colonies of the pathogen were recovered from root lesions. The results showed the susceptibility of the potato variety *S. tuberosum* Group Phureja in this first stage of plant development. According to Dubey *et al*. (2014), *R. solani* is more aggressive in young plants because, in older plants, tissues resist attack. However, new stolons and roots would be re-infected as well as the development of sclerotia in tubers, as observed in the potato crops.

On the other hand, several authors (Dubey *et al.,* 2014; Alban, 2015; Abdel-Sattar *et al*., 2017) suggested differences in pathogenicity or no infection with different isolates within a population, which is apparently related to the state of the potato crops the isolates come from. Genetic aspects, differences in virulence, and AGs present in populations under natural conditions are also factors mentioned by several authors (Carling, 1996; Das *et al.*, 2014), including differences in pathogenicity level related with AGs on different organs of the plant (roots, stems and tubers). This may explain the lower infection observed with six isolates in this research. It is important to mention the efficacy of the *R. solani* inoculation method, using an enriched natural substrate to multiply this type of soil pathogens, such as parboiled rice (Castro *et al*., 2013). Several authors (Castro and Rivillas, 2012; Abdel-Sattar *et al*., 2017; Misawa and Kurose, 2018) suggested other substrates such as wheat, sorghum, carrot, or stems of *Typha latifolia*, since long-term preservation of the inoculum from mycelium in culture medium would affect fungus infectivity.

Finally, the evaluation of symptoms is a topic for future research because of the ability of *R. solani* to cause multi-symptoms. Preliminary pathogenicity verification of isolates was one of the objectives of the present research. However, studies on genetic diversity and AG groups suggest a greater demand in the inoculation standardization, evaluation methods, and experiment design to determine the incidence and severity of isolates on different stages of plant development (Fiers *et al*., 2011). This is also true in the case of evaluations under controlled conditions throughout the crop cycle to find the presence of symptoms and signs (mycelium and sclerotia) in roots, stems and tubers, as suggested by Alban (2015) and Misawa and Kurose (2018). This will allow greater precision in results that determine the presence of pathogenic populations under field conditions.

CONCLUSION

The presence of *R. solani* was verified in all potato producing areas in the Department of Nariño.

Rhizoctonia solani could be isolated from different potato varieties of *S. tuberosum* and *S. tuberosum* Group Phureja.

The *R. solani* isolates showed morphological variability in terms of color, texture and colony growth pattern.

Variability was also noticed in the production and characteristics of the sclerotia and mycelial growth rate.

For the variability of *R. solani*, all isolates were pathogenic, indicating their ability to cause damage in crops.

Conflict of interests: The manuscript was prepared and reviewed with the participation of the authors, who declare that there exists no conflict of interest that puts at risk the validity of the presented results.

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