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FIRST REPORT OF *RHIZOCTONIA SOLANI* ASSOCIATED WITH BLACK SCURF OF POTATO TUBERS IN LESOTHO

[a]Khotsofalang Mothibeli, [a]Moleboheng Lekota, [a]Mpho Liphoto, [a]Motlatsi E. Morojele, [b]Norman Muzhinji
[a] *The National University of Lesotho, Faculty of Agriculture, Department of Crop Science, P. O. Roma. 180. Lesotho.*
[b] *Department of Natural and Applied Sciences, Namibia University of Science and Technology, Windhoek, Namibia.*

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ABSTRACT

Rhizoctonia solani is considered a destructive and widely distributed soil borne pathogenic fungus causing black scurf on potato tubers globally. Morphological, microscopic characteristics and virulence studies were done on ten *R. solani* isolates isolated from four districts of Maseru, Thaba-tseka (Mantsónyane), Quthing and Berea representing three agro-ecological zones; the mountains, lowlands and Senqu river valley. The characteristics include colony colour, hyphal orientation, number of nuclei, and presence of constrictions were studied after five days while colony growth was observed over 24 hour intervals. Variations were observed on all cultural and morphological characters studied in different geographical areas. Among the thirty isolates studied, thirteen of them had pale brown colonies in colour, while eleven had colonies with cream colour, and only 6 isolates had pale to brown coloured colonies. Isolate RB2A had a number of differentiating features such as faster mycelium growth rate of 4 cm at 24 hours, compared to other isolate with 2 cm between 24 hours and 72 hours which progressed to medium (5cm) after 72 hours and then faster after 96 hours. Mycelium growth rate was observed to be independent of the sample collection site (Figure 4; Table 2). The highest nuclei number of 12 was observed with RM3B isolate, while the lowest number of four was observed with RM1A isolate. A pot experiment to evaluate the virulance strenghth of collected isolates was also conducted under greenhouse conditions, with three cultivars commonly grown in Lesotho, inoculated with the isolates collected from different geographical areas were tested. Isolates showed differences in aggressiveness among and within different potato cultivars grown. All the isolates collected were aggressive in causing black scurf of potatoes with percentage disease incidence ranging from 22 to 51 in Fandango, 70 to 92 in Panamera and 4 to 51 in Savannah. However, different cultivars responded diferently to infection by the isolates. Panamera was the most susceptible cultivar with the highest disease incidence of 83% and disease severity index of 85%. This is the first report of *R. solani* causing black scurf on potatoes in Lesotho. Information of *Rhizoctonia sola*ni's prevelence is crucial in the development of effective and timely potato diseases control strategies and growers can make informed cultivar choices for management of *Rhizoctonia solani*.

Corresponding Author: Moleboheng Lekota
Email: lebolek@yahoo.com

INTRODUCTION

The genus *Rhizoctonia* is one of the most important pathogens causing serious damage to a number of crops such as rice (Zheng *et al.*, 2013), wheat and barley (Jaaffar *et al.*, 2016), soybean (Ajayi-Oyetunde and Bradley, 2017), Faba bean (Mostafa and Mohamed, 2018), tomato (Gondal

et al., 2019b) and potato (Gurav *et al.*, 2018; Mejdoub-Trabelsi *et al.*, 2022). All stages of potato development are affected by *R. solani* causing damage to the crop; 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 has black crusts or sclerotia, detracting from quality (Tsror, 2010). Worldwide, this disease causes tremendous decreases in yield and quality and is responsible for losses of up to US$ 75 million/year in several countries (Das *et al.*, 2014).

Rhizoctonia species are classified into three major teleomorphic genera of basidiomycetes that are the *R. solani* complex, inclusive of multinucleate species having the *Thanatephorus* teleomorph, binucleate *Rhizoctonia* species with the *Ceratobasidium* teleomorph and lastly the multinucleate *R. oryzae* and *R. zeae* that possess a *Waitea* teleomorph (Jaaffar, 2012). Identification of *Rhizoctonia* isolates to a taxonomic level is important in studying their epidemiology and control in different cropping systems (Kareem and Hassan, 2018).

Rhizoctonia solani isolates differ in morphology, physiology as well as pathogenic range giving rise to anastomosis groups (Jaaffar, 2012). Several methods have been developed to identify *R. solani* due to its importance as a pathogen to a wide variety of plants. These methods can be used to divide *Rhizoctonia* spp. into more groups. The most commonly used include those that identify the pathogen nuclear status and their anastomisis groups (Kareem and Hassan, 2018). More frequently, *Rhizoctonia* species classification is based on cytomorphology of the hyphae and the culture morphology (Sandoval and Cumagun, 2019). Depending on the nuclear status of the young vegetative hyphae, *Rhizoctonia* isolates can be divided into binucleate and multinucleate groups (Yang, 1994; Kareem and Hassan, 2018). The best known multinucleate plant pathogen is *R. solani* (Kareem and Hassan, 2018). Traditionally, anastomosis groups (AGs) identification has been based on the ability of fungal hyphae to fuse with tester isolates of designated AGs and this has been the commonly used method for assigning isolates to AGs groups (Ferrucho *et al.*, 2012).

Identification of fungi using morphological characteristics are ambiguous and unreliable due to environmental influence hence they require vast experience in the field of classification, especially when closely related fungal groups are studied (Al-Fadhal *et al.*, 2019). Nonetheless, there are various methods that have been introduced for different species of *Rhizoctonia* including biochemical, electrophoretic karyotyping, analysis of genomic fingerprinting, DNA-DNA hybridization and ribosomal analysis (San Aye *et al.*, 2008). Many researchers have shifted to other methods such as polymerase chain reaction (PCR), which is one of the molecular techniques used to target a specific region of the organism's genome that can show the genetic relationships between the fungal isolates to support the morphological identification (Al-Fadhal *et al.*, 2019; Alam *et al.*, 2017). Accurate diagnosis of plant pathogenic fungi is important in designing a rapid and efficient disease management strategy to reduce or prevent damage caused by the fungal infections (Balodi *et al.*, 2017; Al-Fadhal *et al.*, 2019; Bashir *et al.*, 2020).

R. solani has been reported to show a variation in the cultural and morphological characters that can affect management of the disease. Therefore, studies on the variability of *R. solani* isolates are essential for devising effective disease management strategies. The aim of this study was to determine the morphological and microscopic characteristics as well as the virulence of *R. solani* present and prevalent from potato producing areas of different agro-ecological zones in Lesotho.

MATERIALS AND METHODS

Sample Collection

Potato tuber samples showing black scurf symptoms (black spots not easily washed off or brushed off), were obtained from potato fields in three different agro-ecological zones (Table 1; Figure 1). Ten isolates were isolated (two from Berea, three from Quthing, three from Mants'onyane and two from Maseru).

Fungal Isolation

Small pieces of about 10 mm were excised from the margins of infected tubers, then surface sterilised in 1% sodium hydrochlorite (NaOCl) for one minute, rinsed with sterile distilled water and blot dried with paper towel. The isolates were then plated on Potato Dextrose Agar (PDA) in 90mm petri dishes and maintained in an incubator at 25 ºC for seven days. Cultures were then visually and microscopically characterised using cultural morphological markers, icluding colony colour, colony growth rate, hyphal orientation and number of nuclei.

Table 1. Potato isolates and their collection site.

Isolate name	Isolate number	Symptoms on tubers	Origin	Geographical area	Potato cultivar
RM1A	1	Black scurf	Maseru	Lowlands	BP1
RM1B	2	Black scurf	Maseru	Lowlands	BP1
RB2A	3	Black scurf	Berea	Lowlands	Panarema
RB2B	4	Black scurf	Berea	Lowlands	BP1
RM3A	5	Black scurf and elephant hide	Mants'onyane	Mountainous	BP 1
RM3B	6	Black scurf	Mants'onyane	Mountainous	BP 1
RM3C	7	Black scurf	Mants'onyane	Mountainous	Savannah
RQ4A	8	Black scurf and growth cracks	Quthing	Senqu River Valley	Savannah
RQ4B	9	Black scurf and elephant hide	Quthing	Senqu River Valley	Savannah
RQ4C	10	Black scurf, growth cracks and elephant hide	Quthing	Senqu River Valley	Savannah

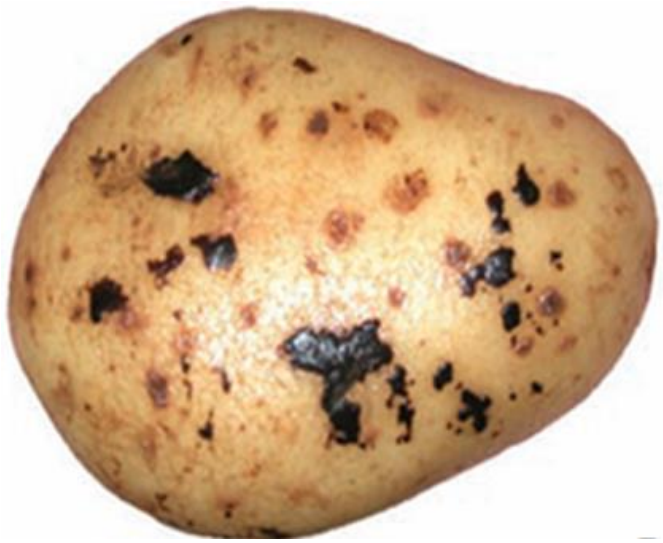

Figure 1. Potato tuber showing black scurf symptoms collected from Mants'onyane.

Morphological Characterization of *Rhizoctonia* Isolates

The isolates were purified by cutting 3 x 3 mm hyphae along with the medium using a sterile scalpel, then sub-cultured on PDA medium in 90 mm petri dishes size. Three replications were made for each isolate. On the fifth day, visual and microscopic morphological characteristics for each isolate were identified based on their appearance on PDA. According to descriptions by Ogoshi (1987) usual characteristics of the *Rhizoctonia* genus include brown pigmented hyphae and constrictions at the base of the branching hyphae, oriented at a right angle from the main hypha. They may also branch at an acute angle. The hyphae are broad and multinucleate, lack clamp connections, and are usually fast growing. Based on these known colony, morphological features as well cellular nuclei for young hyphae, mycelial and sclerotia characteristics and morphology were observed and studied.

Microscopic Charecterization of *Rhizoctonia* Isolates

Microscopic characterisation was done using the method of Gondal *et al.* (2019a) with Nikon SMZ-745T, 100X magnification; slides with mycelium from seven day old cultures stained with lactophenol blue (0.05%) were used to observe morphology of *R. solani* the hypha. Morphology of each culture was compared with previous descriptions (Ogoshi, 1987). After staining the hyphae with Safranin O + a drop of 3% KOH, number of

mycelium nuclei per cell were counted.

Pathogenicity of *Rhizoctonia* Isolates to Three Potato Cultivars

Ten isolates (RM1A, RM1B, RB2A, RB2B, RM3A, RM3B, RM3C, RQ4A, RQ4B and RQ4C) tested for their pathogenicity on three potato cultivars. Isolates were selected for testing based on the visible black scurf symptoms and geographic area representation. The aggressiveness of the isolates to different potato cultivars (Fandago, Panamera and Savannah) was tested in greenhouse conditions at 22 ± 2°C. The tests were performed as previously described by Muzhinji *et al.* (2014). Briefly, PDA plugs of each isolate were added to 500-ml glass conical flasks containing 10 g of sterilized wheat bran moistened with sterile water and incubated for 14 days until fully colonized. Sprouted seed tubers of Fandago, Panamera and Savannah were planted in a 2.5 liter pot containing sterilized sandy-loam soil. Colonized wheat bran (10 g) was uniformly incorporated and mixed with the soil at planting of potato tubers. Control pots were inoculated with sterilized wheat bran only. There were three replicates for each isolate arranged in a randomized complete block design. Each replicate contained four plants. Four plants from each replicate were destructively sampled 120 days after inoculation for black scurf disease index and disease incidence assessment.

Disease severity rating was done according to Woodhall *et al.* (2007) after 120 days of planting. Four plants for each treatment were destructively uprooted and assessed for black scurf symptoms on progeny tubers using the following scale: 0 = no sclerotia present, 1 = less than 1, 2 = 1 to 10, 3 = 11 to 20, 4 = 21 to 50, and 5 = ≥51% of tuber surface area covered in sclerotia. The DSI was calculated as:

$$DSI = \sum[0(n_0) + 0.25(n_1) + 0.5(n_2) + 0.75(n_3) + 1(n_4)] \times 100/(N_{total})$$

Where: n_x = number of tubers in the x rating class and N = total number of tubers in each of the category.

Disease incidence (DI) was also calculated as the percentage of the number of diseased tubers divided by total number of tubers. To fulfil Koch's postulates, re-isolations were performed from progeny tubers showing similar blemishes to the original symptoms harvested from both inoculated and control pots by repeating the same protocols as described above.

Statistical Analysis

The experimental design for the pathogenicity test was a completely randomized design (CRD) with three replications. Results from pathogenicity tests were presented in tabular form to compare blemishes on progeny tubers with those on tubers of origin. Data were subjected to analysis of variance (ANOVA) using the Statistical Analysis Software (SAS) version 9.3 for Windows (SAS Institute 2010). Disease incidence means comparisons were performed using Fisher's Least Significant Difference (LSD) to determine differences in aggressiveness of isolates in causing a certain blemish.

RESULTS

Morphological Characterization of Fungal Isolates

Microscopic observation of the 10 isolates showed a varied morphological variation in colony color, sclerotia size, nuclei number and distribution pattern (Table 2). Diverse sclerotia growth patterns were categorised into abundant, moderate and slight growth patterns irrespective of the isolates collection area (Figure 2; Table 2). Nuclei number for the isolates also varied, with the highest number of 12 observed with RM3B isolate and the lowest number of four observed with RM3B isolate. The 10 isolates showed diversity in pigmentation, seven days after inoculation. The colony colour was observed to be independent of the sample collection site and colony colour was not restricted to a certain isolate. Colony colour was found to be brown, pale brown and cream (Figure 3; Table 2). Most colonies were pale brown in colour (13 colonies), while 11 colonies were cream in colour, with only 6 isolates showed brown colour colonies. The colour of young colonies tended to be white/cream, with colonies turning brown in colour when getting older. Colony colour was found to be inconsistent among and within the three replications. However, isolate 2A had no distinct septa unlike all the isolates. Colony colour for this isolate was also fixed to pale brown in all replications, unlike with other isolates where variation in colony colour was observed.

Colony growth rate was evaluated during the first two days of isolates plating, until when all isolates mycelium filled the 90 cm petri dishes. Colony growth rate was measured at 24 hours' intervals, up to 120 hours (5 days) (Figure 4). Based on the petri dish area covered with the colony, colony diameter was measured, and colony growth was found to be either slow, medium or fast. All the isolates except isolates from RB2A had a slow growth rate (2 cm) between 24 hours and 72 hours

which progressed to medium (5cm) after 72 hours and then faster after 96 hours. Mycelium growth rate was observed to be independent of the sample collection site (Figure 4; Table 2).

Table 2. Morphological characteristics of fungal isolates.

Isolate	Colony colour	Hyphal orientation	Nuclei number	Septa
	Cream	Right angle	4	Present
RM1A	White	Right angle	5	Present
	Brown	Right angle	4	Present
	Cream	Right angle	7	Present
RM1B	Pale brown	Right angle	5	Present
	Pale brown	Right angle	5	Present
	Pale brown	Right angle	6	Indistinct
RB2A	Pale brown	Right angle	5	Indistinct
	Pale brown	Right angle	8	Indistinct
	Pale brown	Right angle	6	Indistinct
RB2B	Pale brown	Right angle	5	Indistinct
	Pale brown	Right angle	8	Indistinct
	Cream	Acute angle	9	Present
RM3A	Pale brown	Acute angle	7	Present
	Pale brown	Acute angle	8	Present
RM3B	Brown	Acute angle	8	Present
	Brown	Acute angle	±10	Present
	Pale brown	Acute angle	12	Present
	Brown	Right angle	9	Present
RM3C	Pale brown	Right angle	6	Present
	Cream	Right angle	9	Present
	Cream	Right angle	6	Present
RQ4A	Pale brown	Right angle	8	Present
	Brown	Right angle	5	Present
	White	Right angle	8	Present
RQ4B	White	Right angle	6	Present
	White	Right angle	9	Present
	Cream	Right angle	8	Present
RQ4C	Cream	Right angle	5	Present
	Cream	Right angle	8	Present

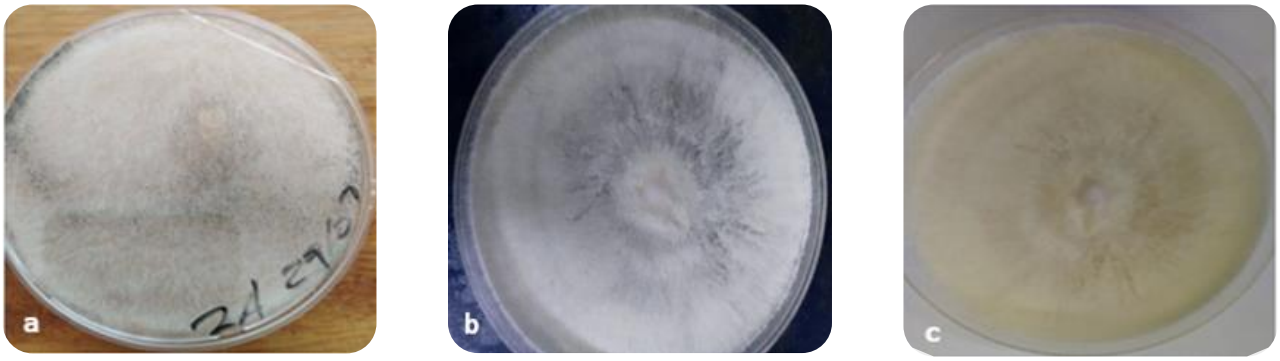

Figure 2. Sclerotia growth pattern for RM3B isolate: (a) Slight growth pattern; (b) moderate growth pattern; (c) abundant growth pattern

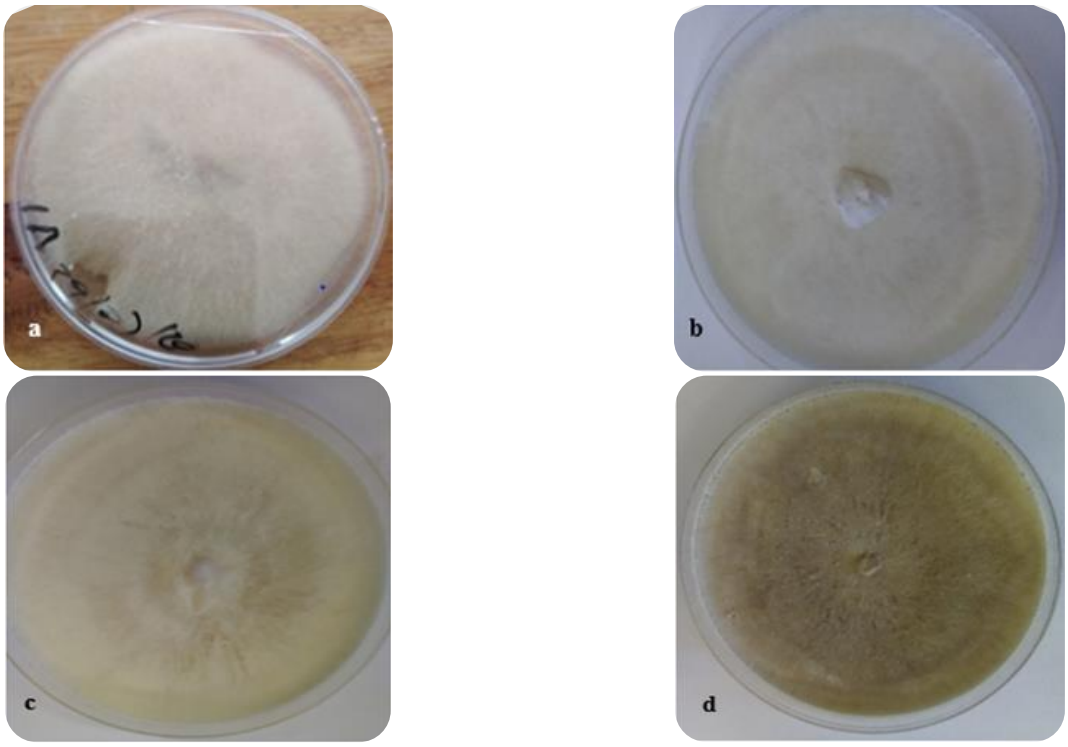

Figure 3. Colony colour (a) White colour, (b) creamy colour, (c) pale brown, (d) brown colour.

Figure 4. Sclerotia growth (a) after 24 hours, (b) after 72 hours, (c) after 120 hours

Microscopic Characterization of Fungal Isolates

Typical characteristics of *R. solani* were observed from all the 10 isolates. Most of the isolates had hyphae with right-angle branches, with few isolates (20%) having hyphae that tanched at acute angle (Figure 5). All isolates had hyphae that branched at the distal septae of cells, dolipore septa, constriction at the junction of these hyphae (Figure 5), and multinucleate hyphal cells (Figure 6; Table 2). Vegetative hyphae were hyaline when young but turned brown with age (Figure 6).

Pathogenicity of *Rhizoctonia solani* Isolates to Potatoes

Pathogenicity and aggressiveness of the 10 isolates to tubers was tested in this study. The results showed that there was a wide variation in aggressiveness among *R. solani* isolates in different potato cultivars in Lesotho. All the isolates tested, were able to cause black scurf on progeny tubers in different cultivars, with DSI ranging from 22 to 51% in Fandango, 70 to 92% in Panamera and 4 to 51% in Savannah (Table 3). Isolate RQ4B was the most aggressive in causing black scurf with the highest severity index in all the three cultivars tested. The results of this study further indicated differing responses of different potato cultivars to different isolates. Panamera was the most susceptible cultivar with the highest severity index scores (Table 3) and the highest disease incidence (Figure 7) in all the ten isolates. On the other hand, Savannah obtained the least severity scores in all the isolates (Table 3).

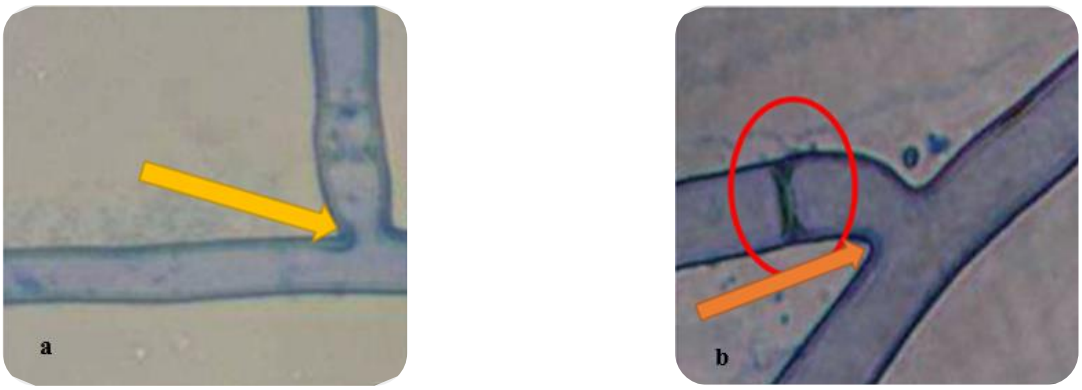

Figure 5. Hyphal cells showing different branch orientation and constrictions (a) hyphae branching at a right angle, (b) hyphae branching at acute angle with constrictions

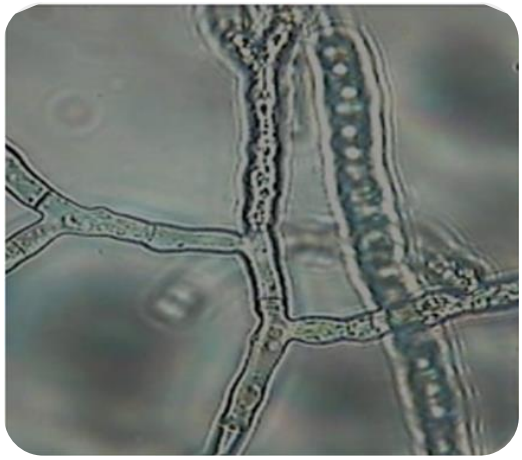

Figure 6. *Rhizoctonia solani* vegetative immature multinucleate hyphae.

Table 3. Disease severity index (%) for black scurf on different potato cultivars.

Isolate	Symptoms	Fandango	Panamera	Savannah
RM1A	Black scurf	51bc[X]	78ab	10ab
RM1B	Black scurf	45ab*	72a	4a
RB2A	Black scurf	30ab	70a	16bc
RB2B	Black scurf	30ab	70a	22ab
RM3A	Black scurf	22a	85ab	12ab
RM3B	Black scurf	39ab	85ab	46cd
RM3C	Black scurf	53ab	76a	23ab
RQ4A	Black scurf	43ab	83ab	19ab
RQ4B	Black scurf	51bc	92bc	51bc
RQ4C	Black scurf	26a	81ab	23bc

DISCUSSION

Morphological characters are the important basic factors for identification of fungal microorganisms and their variability. *Rhizoctonia solani* was prevalent in all the locations in this study, where tubers with black scurf symptoms were collected, indicating the presence of this pathogen in the potato producing areas in Lesotho. Variation in morphological and microscopic characteristics between *Rhizoctonia solani* isolates from different geographical areas has previously been studied by several researcher (Sivalingam *et al.*, 2006; Zhou *et al.*, 2007). Research findings indicated that *R.*

solani consists of many races, forms or groups of various isolates differing in pathogenicity, morphology in culture and/or physiology (Goswami *et al.*, 2010). The result of the present study showed that morphological variation among the *R. solani* isolates existed. Similar observation was noted by other investigators (Pascual *et al.*, 2000; Zhou *et al.*, 2007; Rashad *et al.*, 2012).

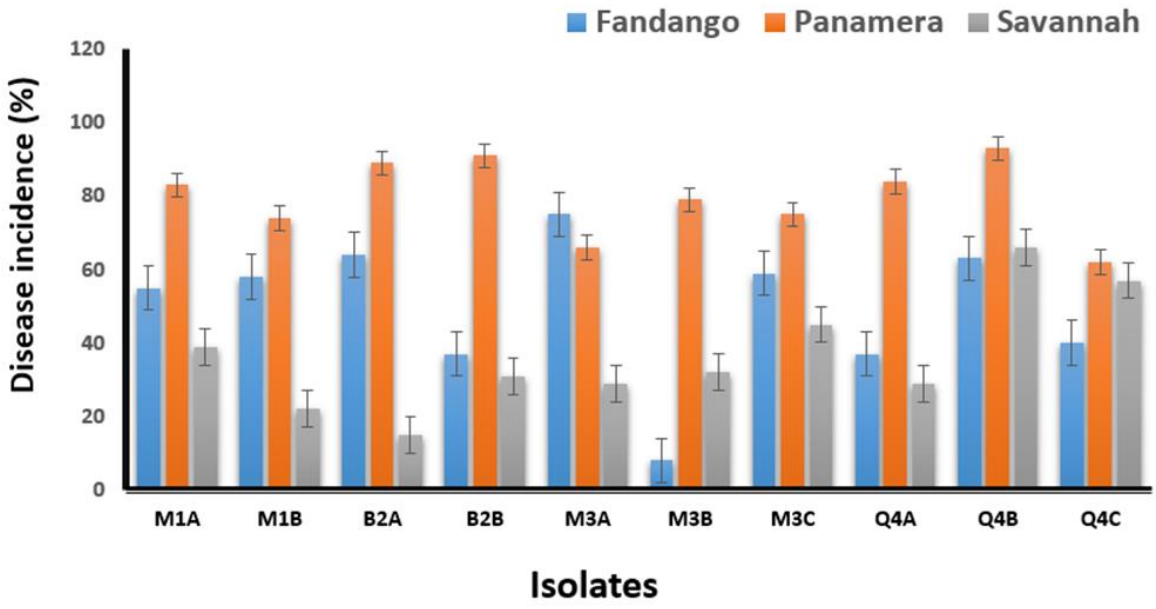

Figure 7. Disease incidence for black scurf disease on different potato cultivars.

Isolates had sclerotia with diverse colours ranging from white, cream, pale brown to dark brown. The results of this study were consistent with the findings by Gondal *et al.* (2019a) who also reported that sclerotia colour may be creamy, pale brown or brown. Moreover, Ajayi-Oyetunde and Bradley (2017) and Desvani *et al.* (2018), observed similar changes in colony colour. Young vegetative hyphae was found to be hyaline but turned brown with aging (Desvani *et al.*, 2018; Ajayi-Oyetunde and Bradley, 2017), this was true with the findings from the current study. Color differences are believed to be caused by differences in pigments produced by the pathogen in the media (Taheri *et al.*, 2007).

Sclerotia growth pattern was determined to differ from isolate to isolate, independent of the collection site. Bintang *et al.* (2017) also found similar results and states that sclerotia growth pattern may vary and is often independent of the collection area of the isolate.

Light microscopy studies revealed that most the isolates of *R. solani* hyphae characteristically branched out at right angle in the distal end of the cell, while few of the isolates had hyphae that branched at an acute angle. These observations were consistent with the findings reported by Al-Fadhal *et al.* (2019). The hyphae of *R. solani* was tubular in shape with septa or partition inside. This was true with Desvani *et al.* (2018), who also observed similar characteristics with *R.solani* isolates collected from potatoes in Indonesia. The currrent study revealed that *R.solani* isolates had septa formed in the branch near the constriction, which was observed as the hyphae matured. Gondal *et al.* (2019a) also found similar results, and states that *R. solani* species are normally characterised by a septum formed in the branch near the constriction, as the hyphae matures. All the hyphal cells observed under the microscope were found to be multinucleate. Similarly, Kanetis *et al.* (2016) and Betancourth-garcía *et al.* (2021) also reported multinucleate hyphal cells after nuclear staining.

In general, there were variations in the morphological and microscopic characteristics among and within the ten collected isolates. This was in agreement with several researchers (Oliveira *et al.*, 2014; Abdel-Sattar *et al.*, 2017), who reported that the variability in colony morphological and microscopic characteristics could be attributed to culture medium, temperature, colony age, host plant species and AG group.

Differences in pathogenicity and aggressiveness of *R solani* isolates were observed in the current study. Several other researchers noted similar findings of differences in pathogenicity or no infection with different isolates (Dubey *et al.*, 2014; Abdel-Sattar *et al.*, 2017). Observations from the current pathogenicity results showed that the 10 isolates had great variability in pathogenicity and virulence, which may be isolate dependent rather than location dependent. However, different isolates showed variability in aggressiveness in different cultivars, implicating that pathogenicity and aggressiveness of the isolates were affected by genotype. Therefore, the effect of cultivar was taken into account in the current pathogenicity testing as it affects virulence of pathogens (Merida *et al.*, 1994; Fiers *et al.*, 2010). Different cultivars show varied levels of resistance to different pathogens (Watkinson *et al.*, 2016). The findings from the current study support this statement since all the isolates were more virulent on cv. Panamera than on cv. Savanna and Fandango on which they caused mild symptoms. Generally, the ability of RQ4B isolates to cause black scurf on potato tubers was higher than other isolates as it was consistent in aggressively causing severe disease in all the three cultivars.

CONCLUSION AND RECOMMENDATION

Rhizoctonia species other than *R. solani* are believed to have little or no role in causing diseases in potato (Woodhall *et al.,* 2007). To our knowledge, this is the first report of *R. solani* causing black scurf of potato tubers in Lesotho. This work has provided improved knowledge about *R. solani* presence found on potato in different locations. Therefore, effective implementation of *Rhizoctonia* disease control strategies on potato using crop rotation and fungicides should be guided by the presence of a particular AG in a particular area. Further research work is needed to evaluate efficacy of fungicides used in control programs and the pathogenicity of different *R. solani* species present in Lesotho. A broader study that could cover all the potato growing areas in Lesotho, for collection of both tuber and soil samples collected over years would give clear insight of areas that have more prevalence of the pathogen, enabling better planning for the management and control of the disease. Different cultivars grown in Lesotho should be considered when collecting samples. Apart from the morphological characterization performed in this study, molecular characterization is recommended to confirm the findings of this study. Farmers should be informed about the presence of this pathogen, as most regard the symptoms as just yet the disease symptoms lead to poor grading of the market ware potatoes and affects seed certification.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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