Anastomosis Groups and Pathogenicity of *Rhizoctonia solani* and Binucleate *Rhizoctonia* from Potato in South Africa

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Abstract

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A survey of anastomosis groups (AG) of *Rhizoctonia* spp. associated with potato diseases was conducted in South Africa. In total, 112 *Rhizoctonia solani* and 19 binucleate *Rhizoctonia* (BNR) isolates were recovered from diseased potato plants, characterized for AG and pathogenicity. The AG identity of the isolates was confirmed using phylogenetic analysis of the internal transcribed spacer region of ribosomal DNA. *R. solani* isolates recovered belonged to AG 3-PT, AG 2-IIIB, AG 4HG-I, AG 4HG-III, and AG 5, while BNR isolates belonged to AG A and AG R, with frequencies of 74, 6.1, 2.3, 2.3, 0.8, 12.2, and 2.3%, respectively. *R. solani* AG 3-PT was the most predominant AG and occurred in all the potato-growing regions sampled, whereas the other AG occurred in distinct locations. Different AG

grouped into distinct clades, with high maximum parsimony and maximum-likelihood bootstrap support for both *R. solani* and BNR. An experiment under greenhouse conditions with representative isolates from different AG showed differences in aggressiveness between and within AG. Isolates of AG 2-IIIB, AG 4HG-III, and AG R were the most aggressive in causing stem canker while AG 3-PT, AG 5, and AG R caused black scurf. This is the first comprehensive survey of *R. solani* and BNR on potato in South Africa using a molecular-based approach. This is the first report of *R. solani* AG 2-IIIB and AG 4 HG-I causing stem and stolon canker and BNR AG A and AG R causing stem canker and black scurf on potato in South Africa.

Potato (*Solanum tuberosum* L.) is one of the most important vegetable crops in the world. In South Africa, it accounts for approximately 60% of the total gross value of vegetables (Potatoes South Africa 2013). The potato crop, as with many other agricultural and horticultural crops, is subject to attack by species of *Rhizoctonia*, which is a globally ubiquitous fungus genus (Sneh et al. 1991).

Rhizoctonia spp. exist as saprophytes, mycorrhizal symbionts, and pathogens of many plant species, and have been reported to cause economically important diseases in more than 250 plant species (Sneh et al. 1991). In potato production, *Rhizoctonia* spp. cause significant quantitative and qualitative yield losses globally (Abd-Elsalam et al. 2009; Campion et al. 2003; Das et al. 2014; Fiers et al. 2011; Tsrör 2010; Woodhall et al. 2008). Marketable yield losses caused by *Rhizoctonia* spp. on potato have been estimated to reach 30% (Banville 1989). *Rhizoctonia* spp. are often associated with the development of sclerotia (black scurf) on progeny tubers, and brown necrotic sunken lesions (cankers) on stems, stolons, and roots in potato production (Banville 1989). In addition, symptoms due to severe infection of the stolons and tubers include cracking, malformation, pitting, desquamation, and elephant hide, leading to tubers of poor marketable quality (Campion et al. 2003; Muzhinji et al. 2014). Formation of aerial tubers,

white collar on the base of stems, purple leaf pigmentation, and stunting of plant shoots are some of the reported aboveground symptoms of *Rhizoctonia* spp. infection of potato (Banville 1989; Tsrör 2010).

In potato production, *Rhizoctonia* spp. are difficult to manage due to their soil- and tuber-borne nature and wide host range. Control and management of *Rhizoctonia* spp. on potato has relied mainly on the use of fungicides but the efficacy of different fungicides varies among and between anastomosis groups (AG) (Campion et al. 2003; Kataria and Gisi 1999; Lehtonen et al. 2008).

Isolates of *Rhizoctonia solani* differ in phenotypic and genotypic characteristics, and have been traditionally arranged in genetically related groups based on hyphal anastomosis grouping. Hitherto, 13 AG (AG 1 to AG 13) have been described, several of which are divided into subgroups on the basis of pathogenicity or genetic characteristics (Carling et al. 2002).

Binucleate *Rhizoctonia* (BNR) are grouped into 21 AG (AG A to AG U), including several subgroups (Sharon et al. 2008; Tsrör 2010). BNR have been reported to be pathogenic to potato in Finland (Lehtonen et al. 2008), Great Britain (Woodhall et al. 2011), the United States (Miles et al. 2013) and China (Yang et al. 2014), although most BNR have been designated saprophytic and biocontrol agents (Carling and Leiner 1986).

Past studies have shown that individual AG have host preferences and are associated with specific disease symptoms. *R. solani* AG 3 is predominantly associated with Solanaceae crops and is subgrouped into AG 3-PT on potato, AG 3-TB on tobacco, and AG 3-TM on tomato (Bartz et al. 2010; Kuninaga et al. 2000). Globally, AG 3-PT has been considered to be the predominant AG most commonly associated with potato diseases and is capable of infecting all subterranean parts of potato plants at any growth stage of the potato crop development (Banville 1989; Tsrör 2010). In addition to *R. solani* AG 3-PT, several other AG have been reported as pathogens of potato, albeit at lower frequencies. AG 2-1 mainly causes stem canker and black scurf (Carling and Leiner 1990; Woodhall et al. 2007); AG 4 causes root, stolon, and tip burning (Anguiz and Martin 1989; Balali et al. 1995); AG 5 mainly causes stem canker and black scurf (Anguiz and Martin 1989; Campion et al. 2003; Truter and Wehner 2004; Woodhall et al. 2008), AG 7 infects shoots and tubers (Abd-Elsalam et al. 2009; Carling et al. 1998), and AG 8 infects roots (Woodhall et al. 2008).

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Delineation of *R. solani* into AG using hyphal interaction has proven to be a questionable and unreliable criterion when it comes to subset identification, especially for subgroups in AG 1 to AG 4 (Carling et al. 2002). Furthermore, some AG have been found to overlap in terms of hyphal fusion (Sharon et al. 2008), and Strausbaugh et al. (2011) noted that reproducibility of AG interactions depends on laboratory conditions and genetic stability. These anomalies demonstrate the limitations of using hyphal anastomosis as the sole criterion for anastomosis grouping. Therefore, grouping of *R. solani* isolates using anastomosis reaction alone cannot provide adequate evidence for placement of an isolate into a subgroup.

Molecular approaches have become increasingly used for accurate identification and placement of isolates into AG in *R. solani* and BNR. The internal transcribed spacer (ITS) region of the ribosomal DNA (rDNA) has often been used for molecular identification of closely related fungi because of the high copy number of rDNA present per genome and the relatively high interspecific and low intraspecific variation of the ITS region (Kuninaga et al. 2000). The ITS region has formally been proposed to the Consortium for the Barcode of Life for adoption as the primary fungal barcode marker (Schoch et al. 2012). It is rapid and less technically challenging than anastomosis grouping by hyphal interactions and has proven to be excellent in resolving between AG and within subgroups (Das et al. 2014; Fang et al. 2013; Sharon et al. 2008; Tsror 2010; Yang et al. 2014).

The main aim of this study was to determine which AG occur in South African potato crops. Although one study (Truter and Wehner 2004) stated that AG 3 is the predominant AG associated with potato diseases in South Africa, no information on the individual subgroups present and limited information on disease symptoms was provided. Therefore, this study aims to use rDNA ITS sequencing to determine

the AG and, where appropriate, the subgroup of *Rhizoctonia* isolates from potato. Isolate diversity was also investigated through analysis of the rDNA ITS sequence. In addition, the aggressiveness of selected isolates of *Rhizoctonia* to potato stems and stolons as well as their ability to cause black scurf in tubers were determined in a greenhouse experiment.

Materials and Methods

Sample collection and fungal isolation. Potato plants and tubers showing typical *R. solani* symptoms (black scurf, elephant hide, and canker on stems, stolons, and roots) and atypical symptoms (malformation, trumpet holes, and cavities on growth cracks) together with potato rhizosphere soils were sampled from various potato-growing regions in South Africa between 2012 and 2014 (Fig. 1). Higher numbers of samples were collected from regions with more intense potato production. For each sample, cultivar name and geographic location under which the potato crops were grown was noted (Table 1).

To obtain *Rhizoctonia* isolates, infected plant material showing visible signs of Rhizoctonia disease symptoms were washed in running tap water and dried in a laminar flow cabinet for 4 h. Small pieces of infected tissue, 4 mm in diameter and 5 mm deep, were excised using a sterile scalpel blade and plated on 90-mm-diameter plates of 1.5% water agar (WA) amended with streptomycin sulfate (Sigma-Aldrich) at 50 mg/liter. The plates were incubated at 25°C for 48 h. Microscopic examination of cultures was carried out and fungal hyphal tips of all isolates that conformed to the *Rhizoctonia* spp. concept according to Carling and Leiner (1990) were transferred to 90-mm-diameter plates containing potato dextrose agar (PDA; Biolab). *Rhizoctonia* isolates were also isolated from rhizosphere soil by first moistening the soil samples with sterile water. Autoclaved beetroot seed were placed as bait in 90-mm petri dishes containing

Fig. 1. Map showing sampling sites and distribution of various *Rhizoctonia* anastomosis groups in major potato-growing regions in South Africa.

Table 1. Binucleate *Rhizoctonia* and *Rhizoctonia solani* isolates collected from South Africa and used in this study

Isolate ^x	AG ^y	Symptom of origin	GenBank accession number	Potato-growing region	Potato variety	Preceding crop	ITS type ^z
Rh1a	3-PT	Black scurf	KJ777545	Gauteng	BP1	Potato	m1
Rh1b	3-PT	Growth crack and elephant hide	KJ777546	Limpopo	BP1	Potato	m2
Rh2a	3-PT	Black scurf	KJ777547	Sandveld	BP1	Unknown	m3
Rh2b	3-PT	Growth crack and elephant hide	KJ777548	Gauteng	BP1	Unknown	m4
Rh2c	3-PT	Black scurf	KJ777549	Northern Cape	BP1	Potato	m4
Rh3a	3-PT	Elephant hide	KF234140	South Western Cape	BP1	Unknown	m5
Rh3c	3-PT	Black scurf	KJ777550	South Western Cape	BP1	Potato	m2
Rh4a	3-PT	Elephant hide	KF234141	South Western Cape	BP1	Unknown	m3
Rh4b	3-PT	Elephant hide	KF234142	South Western Cape	BP1	Unknown	m2
Rh5a	3-PT	Growth crack and Elephant hide	KJ777551	Gauteng	BP1	Unknown	m6
Rh5c	3-PT	Black scurf	KJ777552	Gauteng	BP1	Unknown	m2
Rh6a	3-PT	Elephant hide	KF234143	Sandveld	BP1	Unknown	p1
Rh6b	3-PT	Growth crack and Elephant hide	KF234144	Mpumalanga	BP1	Unknown	m2
Rh7a	3-PT	Black scurf	KJ777553	Mpumalanga	BP1	Wheat	m2
Rh7c	3-PT	Elephant hide	KJ777554	South Western Cape	BP1	Wheat	m7
Rh8b	3-PT	Elephant hide	KJ777555	KwaZulu-Natal	BP1	Unknown	m8
Rh9	3-PT	Black scurf	KJ777556	KwaZulu-Natal	BP1	Unknown	m2
Rh10	3-PT	Growth crack and Elephant hide	KJ777557	Sandveld	BP	Potato	m2
Rh11	3-PT	Black scurf	KJ777558	Sandveld	BP1	Potato	m9
Rh12a	3-PT	Black scurf	KJ777559	Limpopo	BP1	Potato	m2
Rh12b	3-PT	Elephant hide	KJ777560	Limpopo	Mondial	Potato	p2
Rh13	3-PT	Black scurf	KJ777561	KwaZulu-Natal	Mondial	Potato	m2
Rh14a	3-PT	Elephant hide	KJ777562	KwaZulu-Natal	BP1	Potato	m2
Rh14b	3-PT	Black scurf	KJ777563	KwaZulu-Natal	BP1	Potato	p2
Rh17	3-PT	Elephant hide	KJ777566	Sandveld	Nicola	Potato	m2
Rh18	3-PT	Elephant hide	KJ777567	Sandveld	Nicola	Potato	m2
Rh19	3-PT	Elephant hide	KJ777568	Sandveld	Nicola	Potato	m10
Rh24	3-PT	Elephant hide	KJ777573	Sandveld	Nicola	Potato	m11
Rh25	3-PT	Potato rhizosphere soil	KJ777574	KwaZulu-Natal	Unknown	Potato	m12
Rh28	3-PT	Stem canker	KJ777577	Eastern Free State	Mondial	Unknown	m2
Rh29	3-PT	Black scurf	KJ777578	Eastern Free State	BP1	Unknown	m2
Rh32	3-PT	Growth crack and Elephant hide	KJ777581	North West	Mondial	Potato	m13
Rh33	3-PT	Growth crack and Elephant hide	KJ777582	Western Free State	Mondial	Potato	m2
Rh34	3-PT	Growth crack and Elephant hide	KJ777583	Western Free State	Mondial	Potato	m14
Rh35	3-PT	Black scurf	KJ777584	Western Free State	Mondial	Potato	m14
Rh36	3-PT	Black scurf	KJ777585	KwaZulu-Natal	Savanna	Potato	m2
Rh37	3-PT	Potato rhizosphere soil	KJ777586	KwaZulu-Natal	Potato rhizosphere Soil	Potato	m2
Rh38	3-PT	Black scurf	KJ777587	KwaZulu-Natal	BP1	Potato	m15
Rh39	3-PT	Black scurf	KJ777588	KwaZulu-Natal	BP1	Potato	m14
Rh40	3-PT	Black scurf	KJ777589	KwaZulu-Natal	BP1	Potato	m16
Rh41	3-PT	Black scurf	KJ777590	KwaZulu-Natal	BP1	Potato	p3
Rh42	3-PT	Black scurf	KJ777591	South Western Cape	BP1	Potato	m16
Rh43	3-PT	Black scurf	KJ777592	KwaZulu-Natal	BP1	Potato	m17
Rh44	3-PT	Black scurf	KJ777593	South Western Cape	BP1	Potato	m18
Rh45	3-PT	Black scurf	KJ777594	South Western Cape	BP1	Potato	m19
Rh46	3-PT	Black scurf	KJ777595	KwaZulu-Natal	BP1	Potato	m20
Rh47	3-PT	Black scurf	KJ777596	KwaZulu-Natal	BP1	Potato	m24
Rh48	3-PT	Black scurf	KJ777597	Sandveld	BP1	Potato	p4
Rh49	3-PT	Elephant hide	KJ777598	KwaZulu-Natal	BP1	Unknown	m2
Rh50	3-PT	Black scurf	KJ777599	KwaZulu-Natal	BP1	Unknown	p5
Rh51	3-PT	Black scurf	KJ777600	KwaZulu-Natal	BP1	Potato	m2
Rh52	3-PT	Black scurf	KJ777601	KwaZulu-Natal	BP1	Potato	m15
Rh53	3-PT	Black scurf	KJ777602	KwaZulu-Natal	BP1	Potato	m2
Rh54	3-PT	Black scurf	KJ777603	KwaZulu-Natal	BP1	Potato	m2

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^x Isolates with the same numerical value originated from the same tuber but from different blemish types.^y Anastomosis groups.^z Internal transcribed spacer (ITS): m types contain monomorphic sequences and p types contain polymorphic sequences; nd = not determined.

Table 1. (continued from preceding page)

Isolate ^x	AG ^y	Symptom of origin	GenBank accession number	Potato-growing region	Potato variety	Preceding crop	ITS type ^z
Rh55	3-PT	Black scurf	KJ777604	KwaZulu-Natal	BP1	Potato	m14
Rh56	3-PT	Elephant hide	KJ777605	KwaZulu-Natal	BP1	Potato	m2
Rh57	3-PT	Growth crack and Elephant hide	KJ777606	KwaZulu-Natal	Mondial	Unknown	m21
Rh58	3-PT	Elephant hide	KJ777607	KwaZulu-Natal	Mondial	Unknown	m21
Rh59	3-PT	Elephant hide	KJ777608	KwaZulu-Natal	BP1	Unknown	p6
Rh60	3-PT	Elephant hide	KJ777609	KwaZulu-Natal	BP1	Unknown	m2
Rh63	3-PT	Black scurf	KJ777612	Sandveld	Nicola	Potato	m15
Rh64	3-PT	Black scurf	KJ777613	Sandveld	Nicola	Unknown	m22
Rh65	3-PT	Black scurf	KJ777614	Sandveld	Nicola	Unknown	p7
Rh66	3-PT	Black scurf	KJ777615	North West	Mondial	Unknown	m8
Rh67	3-PT	Elephant hide	KJ777616	North West	Mondial	Unknown	p8
Rh68	3-PT	Black scurf	KJ777617	KwaZulu-Natal	Sifra	Potato	m23
Rh69	3-PT	Black scurf	KJ777618	North West	Mondial	Potato	m24
Rh70	3-PT	Black scurf	KJ777619	North West	Nicola	Potato	m2
Rh71	3-PT	Black scurf	KJ777620	KwaZulu-Natal	Mondial	Unknown	m2
Rh72	3-PT	Black scurf	KJ777621	Eastern Free State	Mondial	Potato	m22
Rh73	3-PT	Elephant hide	KJ777622	Eastern Free State	Mondial	Potato	m23
Rh74	3-PT	Elephant hide	KJ777623	KwaZulu-Natal	Mondial	Potato	m2
Rh75	3-PT	Black scurf	KJ777624	KwaZulu-Natal	BP1	Potato	p9
Rh76	3-PT	Black scurf	KJ777625	Sandveld	Fianna	Potato	m2
Rh77	3-PT	Black scurf	KJ777626	North West	Mondial	Potato	m22
Rh78	3-PT	Black scurf	KJ777627	North West	Mondial	Potato	m2
Rh79	3-PT	Black scurf	KJ777628	Western Free State	Mondial	Potato	m2
Rh89	3-PT	Black scurf	KJ777635	Gauteng	BP1	Unknown	m22
Rh92	3-PT	Black scurf	KJ777638	Northern Cape	BP1	Potato	m22
Rh93	3-PT	Black scurf	KJ777639	Northern Cape	BP1	Potato	m2
Rh100	3-PT	Black scurf	KJ777646	Limpopo	BP1	Unknown	m10
Rh101	3-PT	Black scurf	KJ777647	Limpopo	BP1	Unknown	m22
Rh102	3-PT	Black scurf	KJ777648	Limpopo	BP1	Unknown	p10
Rh103	3-PT	Black scurf	KJ777649	Limpopo	BP1	Unknown	m2
Rh104	3-PT	Black scurf	KJ777650	Limpopo	BP1	Unknown	m24
Rh105	3-PT	Elephant Hide	KJ777651	Limpopo	BP1	Unknown	m2
Rh106	3-PT	Growth Crack and Elephant hide	KJ777652	Limpopo	Mondial	Unknown	m2
Rh107	3-PT	Elephant hide	KJ777653	Limpopo	Mondial	Unknown	m2
Rh108	3-PT	Elephant hide	KJ777654	Limpopo	BP1	Unknown	m2
Rh114	3-PT	Black scurf	KJ777660	Sandveld	Nicola	Unknown	m2
Rh115	3-PT	Black scurf	KJ777661	North West	Mondial	Unknown	m2
Rh116	3-PT	Black scurf KJ777662		Limpopo	Nicola	Unknown	m2
Rh117	3-PT	Elephant hide	KJ777663	North West	Mondial	Unknown	m2
Rh118	3-PT	Black scurf	KJ777664	Limpopo	Harmony	Unknown	m2
Rh119	3-PT	Black scurf	KJ777665	North West	Mondial	Unknown	p11
Rh120	3-PT	Black scurf	KJ777666	Mpumalanga	Up-to-Date	Unknown	m2
Rh121	3-PT	Black scurf	KJ777667	Mpumalanga	Up-to-Date	Unknown	m2
PPRI 9806	3-PT	Black scurf	KP298005	Gauteng	Unknown	Unknown	nd
PPRI 9918	3-PT	Black scurf	KP298006	Northern Cape	Unknown	Unknown	nd
PPRI 11952	3-PT	Black scurf	KP298007	Northern Cape	Mondial	Unknown	nd
PPRI 12426	3-PT	Black scurf	KP298008	Unknown	Unknown	Unknown	nd
Rh20	2-IIIB	Stem canker	KJ777569	North West	Mondial	Sugarbeet	nd
Rh21	2-IIIB	Stem canker	KJ777570	North West	Mondial	Sugarbeet	nd
Rh22	2-IIIB	Stem canker	KJ777571	North West	Mondial	Maize	nd
Rh23	2-IIIB	Corky spots	KJ777572	North West	Mondial	Maize	nd
Rh31	2-IIIB	Elephant hide	KJ777580	North West	Mondial	Sugarbeet	nd
Rh97	2-IIIB	Black scurf	KJ777643	Sandveld	BP1	Unknown	nd
Rh99	2-IIIB	Stem canker	KJ777645	North West	BP1	Maize	nd
Rh86	2-IIIB	Stem canker	KJ777632	North West	Mondial	Maize	nd
Rh88	4 HG-I	Stem canker	KJ777634	Gauteng	BP1	Unknown	nd
Rh91	4 HG-I	Stolon canker	KJ777637	Northern Cape	Nicola	Potato	nd
Rh96	4 HG-I	Stem canker	KJ777642	Sandveld	BP1	Unknown	nd
Rh81	4 HG-III	Corky spots	KF712285	Limpopo	Mondial	Soybean	nd
Rh82	4 HG-III	Stem canker	KF712286	Limpopo	Mondial	Soybean	nd
Rh83	4 HG-III	Stem canker	KF712286	Limpopo	Mondial	Soybean	nd
Rh30	5	Black scurf	KJ777579	Sandveld	Fianna	Potato	nd
Rh15	A	Corky spots	KJ777564	Sandveld	Nicola	Unknown	nd

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Table 1. (continued from preceding page)

Isolate ^x	AG ^y	Symptom of origin	GenBank accession number	Potato-growing region	Potato variety	Preceding crop	ITS type ^z
Rh16	A	Elephant hide	KJ777565	Sandveld	Nicola	Unknown	nd
Rh26	A	Growth crack and Elephant hide	KJ777575	Eastern Free State	Mondial	Unknown	nd
Rh27	A	Elephant hide	KJ777576	Eastern Free State	Mondial	Unknown	nd
Rh61	A	Elephant hide	KJ777610	Sandveld	Nicola	Potato	nd
Rh62	A	Elephant hide	KJ777611	Sandveld	Nicola	Potato	nd
Rh80	A	Elephant hide	KJ777629	Western Free State	Mondial	Potato	nd
Rh84	A	Corky spots	KJ777630	Limpopo	Mondial	Soybean	nd
Rh85	A	Corky spots	KJ777631	Limpopo	Mondial	Soybean	nd
Rh87	A	Elephant hide	KJ777633	Gauteng	BP1	Potato	nd
Rh90	A	Black scurf	KJ777636	Sandveld	Nicola	Unknown	nd
Rh94	A	Black scurf	KJ777640	Northern Cape	BP1	Potato	nd
Rh95	A	Black scurf	KJ777641	Sandveld	BP1	Potato	nd
Rh98	A	Elephant hide	KJ777644	Limpopo	BP1	Maize	nd
Rh109	A	Elephant hide	KJ777655	Limpopo	BP1	Unknown	nd
Rh110	A	Elephant hide	KJ777656	Sandveld	Nicola	Unknown	nd
Rh111	R	Elephant hide	KJ777657	Sandveld	Nicola	Unknown	nd
Rh112	R	Black scurf	KJ777658	Sandveld	Nicola	Unknown	nd
Rh113	R	Black scurf	KJ777659	Sandveld	Nicola	Unknown	nd

Table 2. Binucleate *Rhizoctonia* (BNR) and *Rhizoctonia solani* reference isolates used in phylogenetic analysis on ITS sequences

Isolate number	Anastomosis group	Host plant or substrate	Geographic origin	GenBank accession number
BNR				
1Fuk-600 ^y	A	Rose	Japan	AB196663
4Oit-800 ^y	A	Rose	Japan	AB196661
AH-1 ^y	A	Peanut	Japan	AB196639
C-538 ^y	A	Potato	Japan	AB196640
GZ-7	A	Potato	China	KF626386
GZ-11	A	Potato	China	KF626390
HL-2-2	A	Potato	China	KF176573
HL-20-1	A	Potato	China	KF176576
HuB-1	A	Potato	China	KF176578
JL-5-1	A	Potato	China	JX885460
LN-6	A	Potato	China	KF176596
Rh110	A	Potato rhizosphere soil	Italy	EF017213
SC-2	A	Potato rhizosphere soil	China	KF626393
SD-1-1	A	Potato	China	KF176601
SD-9-1	A	Potato	China	KF176605
SX-3	A	Potato	China	KF176607
RU56-8 ^y	A	Soil	United States of America	DQ102417
AH-2	Fa (F) ^z	Potato	China	KF176561
HuB-6	Fa (F) ^z	Potato	China	KF176581
HuN-1-1	Fa (F) ^z	Potato	China	KF176582
PS-17 ^y	Fa	Pea	Japan	AB219144
SX-2	Fa (F) ^z	Potato	China	KF176606
Str10 ^y	Fa	Strawberry	Israel	DQ102434
Str36 ^y	Fa	Strawberry	Israel	DQ102435
AH-6 ^y	Fb	Peanut	Japan	AB196645
Bn38 ^y	Fb	Soybean	United States of America	AF354081
FKO2-28 ^y	Fb	Soil	Japan	AB219145
55D21 ^y	I	Sugar beet	Japan	AB290023
AV-2 ^y	I	<i>Artemisia</i> sp.	Japan	AB196650
HuN-4-1	I	Potato	China	KF176586
Im2 ^y	I	Strawberry	United States of America	DQ102444
R92	I (unknown) ^z	Potato	Finland	DQ913035
206	K (A) ^z	Potato	United States of America	KC782951
56D17 ^y	K	Sugar beet	Japan	AB286932
AC-1 ^y	K	Onion	Japan	AB122145
GS-19	K	Potato	China	KF176568
HeB-16	K	Potato	China	KF176571
NM-19-1	K	Potato	China	KF176597

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^y BNR tester isolates recommended by Sharon et al. (2008).^z Original assigned anastomosis group indicated in parentheses.

soil samples and incubated for 48 h at 25°C. After incubation, bait seed were transferred from soil to 1.5% WA amended with streptomycin at 50 mg/liter, incubated, and purified as described for plant material. Purified cultures were maintained on colonized barley grains at 4°C for further studies. Reference isolates of AG 3-PT, obtained from the National Collection of Fungi, hosted by the Plant Protection Research Institute, Agricultural Research Council, South Africa, were also included in the study.

Genomic DNA extraction. To extract total genomic DNA, isolates were subcultured on PDA for 7 days at 25°C. Fungal mycelia were scraped from PDA plates and transferred to 2-ml Eppendorf tubes. Total genomic DNA was extracted from mycelia of all isolates of *R. solani* and BNR using the ZR soil microbe DNA kit (Zymo Research Corporation) according to the manufacturer's protocol recommendations. The concentration and quality of extracted DNA was determined by NanoDrop UV spectrophotometry (NanoDrop Technologies).

Polymerase chain reaction amplification and sequencing. The ITS region for each *R. solani* and BNR isolate was amplified using the universal primers ITS1-F (5'-CTTGGTCATTTAGAGGAAGTAA-3') (Gardes and Bruns 1993) and ITS-4 (5'-TCCTCCGCTTATTGATATGC-3') (White et al. 1990) that anneal to the flanking 18S and 28S rRNA genes. Amplification was conducted in 50-μl

reaction mixtures containing 4 ng of template DNA; 250 μM each dATP, dTTP, dGTP, and dCTP (Bioline); 10× NH₄ reaction buffer, consisting of 160 mM (NH₄)₂SO₄, 670 mM Tris-HCl at pH8.8, and 100 mM KCl (Bioline); 0.25 U of BIOTaq DNA polymerase (Bioline); 3 mM MgCl₂; and 0.2 μM each primer. Amplification was carried out in a thermal cycler (Bio-Rad) with the following conditions: an initial step at 95°C for 3 min; followed by 35 cycles at 95°C for 30 s, 55°C for 30 s, and 72°C for 45 s; and a final extension of 72°C for 5 min. A 5-μl aliquot of each polymerase chain reaction (PCR) product was separated by electrophoresis on 1.5% (wt/vol) Metaphor agarose (Lonza) stained with ethidium bromide solution (0.1 mg/liter) and visualized using a UV transilluminator. When the bands of the appropriate size (700 bp) were observed, the remaining PCR product was purified using Sephadex spin column (5 g of Sephadex G-50 powder dissolved in 75 ml of sterile water) sequenced in both directions using the PCR primers and the BigDye Terminator v.3.1. Cycle Sequencing Kit (Applied Biosystems). The resulting amplicons were analyzed with ABI3500xl model genetic analyzer (Applied Biosystems) at the University of Pretoria. Consensus sequences were created from forward and reverse sequences using BioEdit v 7.1.3 and manually edited for base mismatches when required. Sequences generated in the present study have been deposited in GenBank (Table 1).

Table 2. (continued from preceding page)

Isolate number	Anastomosis group	Host plant or substrate	Geographic origin	GenBank accession number
SH-10 ^y	K	Soil	Japan	AB196652
SX-7	K	Potato	China	KF176609
Bn-37 ^y	R	Cucumber	United States of America	AB219146
RhJN207yWCz3	R	Azalea	United States of America	HQ269817
RhMY074WAZ3	R	Azalea	United States of America	HQ269823
WUF-ST-Rhwf3	R	Strawberry	Australia	DQ885780
X4-3 ^y	R	Ginger	China	AB286942
Xb-1-3 ^y	R	<i>Betula</i> sp.	China	DQ885784
HuN-3	U	Potato	China	KF176585
MWR-20 ^y	U	Rose	Japan	AB196664
MWR-22 ^y	U	Rose	Japan	AB196666
MWR-24 ^y	U	Rose	Japan	AB196665
cc43	Unknown	Potato	United Kingdom	FR828480
<i>R. solani</i>				
DB13MAG05	2-2IIIB	<i>Rebutia perplexa</i>	Italy	KF719318
F16	2-2IIIB	Beetroot	United States of America	FJ492075
F514	2-2IIIB	Beetroot	United States of America	FJ492150
F521	2-2IIIB	Beetroot	United States of America	GU811674
SJ07	2-2IIIB	Soybean	Brazil	AY270015
660	3-PT	Potato	Brazil	AB019011
AK0503 11S	3-PT	Potato	Switzerland	EF370445
CP245	3-PT	Potato	United States of America	AB019013
MIAE00082	3-PT	Potato	France	HQ898689
MIAE00152	3-PT	Potato	France	HQ898684
O1-1	3-PT	Tomato	Japan	AB547393
P42	3-PT	Potato	United States of America	AB547385
R120	3-PT	Potato	Finland	DQ913031
RS027-1	3-PT	Potato	New Zealand	JX161945
RS054-3	3-PT	Potato	New Zealand	JX161928
T54	3-PT	Potato	Spain	AY387534
T96	3-PT	Potato	Spain	AY387565
AG4 tester	4HG-I	Unknown	Unknown	JX162012
RS12	4HG-I	<i>Arachis hypogaea</i>	Argentina	JQ616861
YR-7	4HG-I	Maize	China	HQ636465
JWC-13	4HG-III	Potato	China	JQ995157
RAPS3	4HG-III	Soybean	India	JF701709
RS72	4HG-III	Soil	Argentina	JQ616873
SR-26	4HG-III	Spinach	China	KF857546
R48	5	Potato	France	DQ355140
R206	5	Lupin	Canada	EU730866
Rs2010A-W	5	Wheat	Great Britain	HE667746
Y55	5	Potato	Great Britain	DQ355139

Phylogenetic analysis. Consensus sequences were compared with the nucleotide databases in GenBank (<http://www.ncbi.nlm.nih.gov/genbank/>) and MycoBank (www.mycobank.org) using BLAST to find the closest match on maximal percentage identity. Reference sequences (Das et al. 2014; Fang et al. 2013; Fiers et al. 2011; Sharon et al. 2008; Woodhall et al. 2007, 2012; Yang et al. 2014) for the different AG were imported from GenBank and included in the phylogenetic analysis (Table 2). Multiple sequence alignments were generated with MAFFT v.7 (<http://mafft.cbrc.jp/alignment/server/index.html>). Gaps were treated as missing data in the subsequent analyses. Phylogenetic analyses were done separately for *R. solani* and BNR datasets and were based on maximum parsimony (MP) using

Phylogenetic Analysis Using Parsimony* and Other Methods, version 4 (PAUP 4.0*) (Swofford 2000) and maximum likelihood (ML) analyses using PhyML 3.0 (Guindon and Gascuel 2003). Heuristic searches in MP were performed with random addition of sequences (100 replicates), tree bisection-reconnection branch swapping, MULPAR effective, and MaxTrees set to auto-increase. The consistency and retention indices (CI and RI, respectively) were determined for the data sets. Bootstrap analyses were performed to determine branching point confidence intervals (1,000 replicates) for the most parsimonious trees generated for each data set. Selection of models of nucleotide substitution for the PhyML analyses, implementing the Akaike information criterion, was determined with

Table 3. Incidence of individual anastomosis groups (AG) of *Rhizoctonia solani* and binucleate *Rhizoctonia* isolates collected from each symptom

Symptom of origin	AG3-PT	AG 2-IIIB	AG 4HG-I	AG 4HG-III	AG 5	AG A	AG R	Total
Black scurf	40	1	0	0	1	3	2	47
Stem canker	1	5	2	2	0	0	0	10
Stolon canker	0	0	1	0	0	0	0	1
Elephant hide	25	1	0	0	0	9	1	36
Growth crack and Elephant hide	10	0	0	0	0	1	0	11
Potato rhizosphere soil	2	1	0	0	0	0	0	3
Corky spots	0	0	0	1	0	3	0	4
Total	97	8	3	3	1	16	3	131

Table 4. Variable sequence regions and location of polymorphic in the internal transcribed spacer (ITS) region of *Rhizoctonia solani* AG3-PT isolates^z

	ITS																								5.8S
Sequence	18	19	22	23	29	32	33	37	39	40	43	76	89	90	104	112	116	120	146	148	157	158	159	369	
m1	1	G	T	C	A	C	C	C	T	C	T	A	T	T	G	G	T	C	A	A	T	T	T	C	
m2	42	G	T	C	A	C	C	C	T	C	C	C	A	T	T	G	G	T	C	A	A	T	T	T	C
m3	2	T	-	C	A	C	C	C	T	C	C	C	A	T	T	G	G	T	C	A	A	T	C	A	C
m4	2	G	T	C	A	C	C	C	T	C	C	C	A	T	T	G	G	T	C	A	A	T	T	T	-
m5	1	G	T	C	A	C	C	C	T	C	C	C	A	T	T	G	G	T	C	A	A	T	T	T	C
m6	1	G	T	C	A	C	C	C	T	C	C	C	A	T	T	G	G	T	C	A	A	T	T	T	-
m7	1	G	T	G	C	C	C	C	T	C	C	C	A	T	T	G	G	T	C	A	A	T	T	T	C
m8	2	G	T	C	A	C	C	C	T	C	C	C	A	T	T	G	A	T	C	A	A	T	T	T	C
m9	1	G	T	C	A	C	T	C	T	C	C	C	A	T	A	A	G	C	C	A	C	T	T	T	C
m10	2	G	T	C	A	C	A	C	T	C	C	C	A	T	T	A	A	T	C	A	A	T	T	T	C
m11	1	G	T	C	A	C	C	C	T	C	C	C	A	T	T	G	G	T	C	A	A	T	T	T	C
m12	1	G	T	C	A	C	C	C	T	C	C	C	A	T	T	G	G	T	C	A	A	T	T	T	C
m13	2	G	T	C	A	C	C	C	T	C	C	C	A	T	T	G	G	T	C	A	A	T	T	A	C
m14	3	G	T	C	A	C	C	C	T	C	C	C	A	T	T	G	G	T	C	A	A	T	C	T	C
m15	3	G	T	C	A	C	C	C	T	C	C	C	A	T	A	G	G	T	T	A	A	T	T	T	C
m16	2	G	T	C	A	C	C	C	T	C	T	C	A	T	T	G	A	C	T	A	A	T	T	T	C
m17	1	G	T	C	A	C	A	C	T	C	C	G	T	T	T	A	G	T	T	A	A	T	T	T	C
m18	2	G	T	C	A	C	A	C	T	C	C	C	A	T	T	G	G	T	T	A	A	T	T	T	C
m19	2	G	T	C	A	C	T	C	T	C	C	C	A	T	A	A	G	C	T	C	C	T	T	T	C
m20	4	G	T	C	A	C	T	T	T	C	C	C	A	G	A	G	G	T	T	A	A	T	T	T	C
m21	1	G	T	C	A	C	A	C	C	C	C	C	A	T	T	G	A	T	T	A	A	T	T	T	C
m22	2	G	T	C	A	C	A	C	C	C	C	C	A	T	T	G	A	T	T	A	A	T	T	T	C
m23	2	G	T	C	A	C	C	C	T	C	C	C	A	T	T	G	G	T	T	A	A	T	T	T	C
m24	4	G	T	C	A	C	T	C	T	C	C	C	A	T	A	C	C	T	T	T	A	T	T	T	C
p1	1	G	T	C	A	C	C	C	T	C	C	C	A	T	T	G	G	T	C	A	A	W	T	T	C
p2	2	G	T	C	A	C	C	C	T	C	C	C	A	T	T	A	R	T	C	A	A	T	T	T	C
p3	1	G	T	C	A	C	C	C	T	C	C	C	A	T	T	G	R	T	T	A	A	T	T	T	C
p4	1	G	T	C	A	C	C	C	T	C	C	C	A	T	T	G	G	T	T	A	A	W	T	T	C
p5	1	G	T	C	A	C	C	C	T	C	C	C	A	T	T	G	G	T	T	A	A	W	T	T	C
p6	1	G	T	C	A	C	Y	C	T	C	C	C	A	T	W	R	G	Y	T	A	M	T	T	T	C
p7	1	G	T	C	A	C	A	C	C	C	C	C	A	T	T	G	G	T	T	A	A	W	T	T	C
p8	1	G	T	C	A	C	A	C	C	C	C	C	A	T	T	G	G	T	T	A	A	T	T	T	C
p9	1	G	T	C	A	C	C	C	T	C	C	C	A	T	T	G	G	T	T	A	A	W	T	T	C
p10	1	G	T	C	A	C	C	C	T	C	C	C	A	G	W	R	G	T	T	A	M	T	T	T	C
p11	1	G	T	C	A	C	C	C	T	C	C	C	A	T	T	G	G	T	T	A	A	T	T	T	C

(continued on next page)

^z M and P followed by a number designate the different monomorphic and polymorphic sequences. Nucleotide numbering is the same as used by Fiers et al. (2011).

jModeltest 2.1.7 (Darriba et al. 2012; Guindon and Gascuel 2003). A TPM2uf+I+G model and TIM3+G model were used for the *K. solani* and BNR datasets, respectively, and 1,000 ML bootstrap replicates were conducted. *Athelia rolfssii* (AY684917) was used as outgroup in all the analyses (Fang et al. 2013). All phylogenetic trees were graphically edited with FigTree v1.3.1 (<http://tree.bio.ed.ac.uk/software/figtree/>).

Aggressiveness of isolates to potato. In all, 25 isolates belonging to different AG—AG 3-PT (Rh28, Rh35, Rh36, Rh37, Rh42, Rh44, Rh45, Rh46, Rh54, Rh69, Rh71, Rh77, Rh89, and Rh102), AG 2-IIIB (Rh20, Rh23, and Rh86), AG 4HG-I (Rh88 and Rh91), AG 4HG-III (Rh81, Rh82, and Rh83), AG 5 (Rh30), AG A (Rh80), and AG R (Rh113)—were selected and tested for their pathogenicity on potato. Isolates were selected for testing based on AG, symptom of origin, and potato-growing region representation.

The aggressiveness of the isolates to potato ('Mondial') was tested in greenhouse conditions at $25 \pm 2^\circ\text{C}$. The tests were performed as previously described by Muzhinji et al. (2014). Briefly, PDA plugs of each isolate were added to 250-ml glass jars containing 10 g of sterilized barley grains moistened with sterile water and incubated for 14 days until fully colonized. A sprouted seed tuber of Mondial was planted in a 5-liter pot containing sterile potting mixture of pine bark, sand, and soil at 1:1:1 (wt/wt). Colonized barley grains (10 g) were placed 10 mm above the tuber and covered with a 10-mm layer of sand. Control pots were inoculated with sterile barley grains only. There were three replicates for each isolate arranged in a randomized

After 120 days, the remaining plants were removed 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 = $\geq 51\%$ of tuber surface area covered in sclerotia. The DI was calculated as $DI = \sum [0(n_0) + 0.25(n_1) + 0.5(n_2) + 0.75(n_3) + 1(n_4)] \times 100 / (N_{\text{total}})$, where n_x = number of tubers in the x rating class and N = total number of tubers in each of the category. To fulfill Koch's postulates, isolations were made from infected tissues and resulting fungi identified as described above.

Statistical analysis. The experimental design for the pathogenicity test was a complete randomized block design with three replications. Data were subjected to analysis of variance and treatment means were separated by Duncan's multiple range test ($\alpha = 0.05$).

Table 4. (continued from preceding page)

The data were analyzed using GENSTAT 14th Edition (VSN International).

Results

Collection and identification of *Rhizoctonia* isolates. In total, 131 isolates conforming to the *Rhizoctonia* sp. concept were obtained from 10 potato-growing regions in South Africa (Table 1). All isolates retrieved had white to brown mycelium with typical right-angle branches at the distal septae of cells. Most isolates ($n = 112$) were multinucleate and identified as *R. solani*, whereas 19 isolates were binucleate. Assignment of isolates to the various AG were based on BLAST and phylogenetic analysis of the ITS region. Among the 112 *R. solani* isolates, 97, 8, 3, 3, and 1 isolates belonged to AG 3-PT, AG 2-IIIB, AG 4HG-III, AG 4HG-I, and AG 5, respectively. For BNR, 16 isolates belonged to AG A and 3 belonged to AG R.

Most of the AG 3-PT isolates (41%) and the only AG 5 isolate were obtained from sclerotia, whereas most of AG 2-IIIB (63%), AG 4HG-I (67%), and AG 4HG-III (67%) isolates in this study were obtained from stem canker (Table 3). Some AG 3-PT, AG 2-IIIB, and AG 4HG-III and most AG A (81%) isolates were also obtained from other symptoms on tubers such as elephant hide, elephant hide in growth crack cavities, and corky spots. Two AG 3-PT isolates were recovered from potato rhizosphere soil.

AG 3-PT isolates were widely distributed and were recovered from all of the potato-growing regions sampled (Fig. 1). AG 2-IIIB isolates were recovered from two growing regions, (North West and Sandveld) while AG 4HG-III was obtained from one growing region (Limpopo). BNR AG A was widely distributed, occurring in six growing regions sampled, while AG R isolates were obtained from the Sandveld.

PCR, sequencing, and phylogenetic analysis. PCR amplification of the DNA of *R. solani* and BNR isolates retrieved from potato in South Africa using universal primers ITS1-F and ITS-4 generated a fragment approximately 700 bp long for each isolate. Sequencing of the ITS PCR products revealed sequence heterogeneity (presence of two or more overlapping peaks in the electrophoregrams) in 12 *R. solani* isolates. In all, 11 polymorphic sequence types (p1 to p11) and 24 monomorphic sequence types (m1 to m24) were observed for AG 3-PT (Table 4). Sequence variation occurred at 23 base positions in the ITS 1 region and at 14 base positions in the ITS 2 region. The 5.8S rDNA gene sequence was highly conserved among isolates. Only three AG 3-PT isolates had missing bases in the 5.8S rDNA gene (Table 4).

The ITS sequences of all the multinucleate and BNR isolates (Table 1) were used in MP and ML analysis together with reference sequences retrieved from GenBank (Table 2). Separate datasets were compiled for *R. solani* and BNR isolates. The dataset for *R. solani* included 146 taxa and sequences were trimmed to 663 bp to remove primer bases before alignment. MP analysis included 156 parsimony-informative characters and the tree length was 367, CI was 0.6621, and FI was 0.9404. The tree topology was mostly the same for the MP and ML analysis (results not shown), except for the AG 5 clade that formed a sister group with AG 3-PT in the ML analysis, whereas AG 5 formed a sister clade with AG 2-IIIB in the MP analysis. The isolates from South Africa grouped into five distinct clades corresponding to AG 3-PT, AG 5, AG 2-IIIB, AG 4HG-III, and AG 4HG-I (Fig. 2).

The BNR dataset contained 69 taxa and was trimmed to 658 bp before alignment. MP analysis included 203 parsimony-informative characters and the tree length was 512, CI was 0.6211, and RI was 0.9246. The MP and ML analysis resulted in the same tree topology (results not shown), except for the AG K isolates that grouped as a subclade within the AG A clade in the ML analysis, compared with a separate clade in the MP analysis. The BNR isolates from South Africa grouped into two main clades, corresponding to AG A and AG R (Fig. 3). Reference isolates from potato grouped with AG A, AG K, AG Fa, AG U, and AG I. One isolate from potato (KC782951) in the United States was reported as AG A (Miles et al. 2013) but, according to our analysis, it belonged to AG K. The unknown AG from potato sprouts reported from Finland (Lehtonen et al. 2008) grouped with AG I in our analysis. There was no relationship

between geographic origin and clades on the phylogenetic trees because most of the isolates within the larger clades (e.g., AG 3-PT and AG A) represented most of the sampling areas.

Aggressiveness of isolates to potato. Testing in the greenhouse for aggressiveness to stems and tubers with representative AG isolated in this study showed that there was an inter- and intravariation in aggressiveness of *Rhizoctonia* AG associated with potato diseases in South Africa. All AG 3-PT isolates tested, except Rh35 and Rh77, were able to cause black scurf on progeny tubers, with DI ranging from 17 to 41% (Table 5). A wide variation in aggressiveness toward potato was observed among AG 3-PT isolates, as reflected in DI ranging from 0 to 41% for black scurf and 0 to 63% for stem canker. An isolate of AG 5 caused black scurf but the severity of the disease was minor in comparison with disease from AG 3-PT isolates. AG 2-IIIB, AG 3-PT, AG 4HG-III, AG A, and AG R were aggressive on stems causing stem canker and AG 3-PT was more aggressive on stolons causing stolon canker than any other AG. AG 4HG-I was able to cause mild stem and stolon canker. AG R was capable of causing black scurf and stem canker, with DI of 27 and 44%, respectively, whereas AG A was able to cause mild stem canker and black scurf on potato (Supplementary Fig. S1).

Discussion

In this study, a survey of AG of *R. solani* and BNR associated with potato diseases was carried out in South Africa. Previously, Truter and Wehner (2004) characterized *R. solani* isolated from potato diseases in South Africa using hyphal interactions with tester isolates. Although the classical hyphae fusion is still valid and supported by the use of DNA-based molecular methods, the anastomosis reaction has not always been straightforward and has proven to be unreliable in some AG and in subset identification (Fang et al. 2013; Sharon et al. 2008). Furthermore, reproducibility of AG interactions can be affected by factors such as laboratory environment, nutritional conditions, and genetic stability (Carling et al. 2002).

Sequencing and phylogenetic analysis of the ITS region has been confirmed to reliably divide isolates of *R. solani* and BNR into distinct clades, which correspond to the different AG and subgroups (Fang et al. 2013; Kuninaga et al. 2000; Sharon et al. 2008; Tsror 2010). According to ITS sequences, *R. solani* isolates retrieved from different potato diseases in South Africa were predominantly AG 3-PT (74%). These results conform to global reports and previous research confirming the predominance of AG 3-PT on potato crops (Anguiz and Martin 1989; Balali et al. 1995; Campion et al. 2003; Carling and Leiner 1986; Das et al. 2014; Fiers et al. 2011; Truter and Wehner 2004; Woodhall et al. 2007). In addition to its evidently high virulence on potato, one of the reasons why AG 3-PT is so common in potato is its ability to form sclerotia (black scurf) on tubers. The results are in accordance with the observation of Lehtonen et al. (2009), who postulated that AG 3-PT is much more efficient in producing sclerotia on tubers than other AG, reflecting a highly specialized nature of AG 3-PT in infecting potato.

In this study, in addition to AG 3-PT, members of other *R. solani* AG (AG 2-IIIB, AG 4HG-I, AG 4HG-III, and AG 5) were retrieved from different *Rhizoctonia* disease symptoms, albeit with lower frequencies. This study showed that AG 3-PT, AG 5, and AG R are implicated in causing black scurf on potato tubers in South Africa. AG 3-PT and AG 5 were also identified and found to be associated with black scurf on potato in France (Campion et al. 2003), the United Kingdom (Woodhall et al. 2007), and South Africa (Truter and Wehner 2004).

The majority of AG 2-IIIB isolates (88%) were found in samples from the North West growing region. The localized occurrence of AG and subgroups in distinct growing regions may be due to a susceptible preceding crop. Its occurrence on potato in this region may be attributed to maize used as a preceding crop (Table 1). AG 2-IIIB has been reported to cause crown and root rot of sugar beet, maize, and soybean (Liu and Sinclair 1991). The susceptibility of maize to *R. solani* AG 2-IIIB has been demonstrated (Kluth et al. 2010).

AG 4HG-III was found exclusively in the growing region Limpopo, where soybean was the preceding crop. The effect of AG 4HG-III on potato stems was recently reported for the first time in

Fig. 2. One of the most parsimonious trees based on ribosomal DNA internal transcribed spacer sequences of 146 *Rhizoctonia solani* isolates. Maximum parsimony (MP) and maximum likelihood (ML) bootstrap support values $\geq 70\%$ from 1,000 replicates are given at the nodes. Thickened lines indicate MP and ML of 100% each. The tree was rooted to *Athelia rolfsii*.

South Africa by Muzhinji et al. (2014). AG 4HG-III was isolated from a potato field that was previously planted to soybean (Table 1). Therefore, this might suggest that crop rotation may have an influence on the incidence and composition of the *Rhizoctonia* AG recovered from the potato cropping system.

AG 2-1 was not detected in this study despite the fact that they it has been previously reported to be associated with potato in other countries (Anguiz and Martin 1989; Balali et al. 1995; Campion et al. 2003; Das et al. 2014; Woodhall et al. 2007). Although AG 7 has been reported from soil in which potato plants were grown in South Africa, its effect on potato was not demonstrated (Truter and Wehner 2004). The variability in the range of AG present in potato fields in different countries could be related to selection factors that favor the occurrence of certain AG over others; for example, the species used for crop rotation and prevailing environmental conditions. The presence of AG 3-PT and AG 4 has been related in some reports

to certain environmental conditions such as temperature, with AG 4 being more prevalent under warm and moist conditions and AG 3-PT most virulent in cool growing conditions (Anguiz and Martin 1989; Campion et al. 2003).

Though previous research has shown that binucleates can be pathogenic to stems or tubers (Miles et al. 2013; Yang et al. 2014), they were isolated from tuber blemishes in this study. The BNR isolated in this study belonged to AG A and AG R. This research supports previous observations that *Rhizoctonia* disease in potato crops could be caused by several AG of BNR.

Kuninaga et al. (2000) and Sharon et al. (2008) demonstrated that grouping of *R. solani* based on ITS sequencing supports the grouping of *R. solani* isolates based on hyphal AG. This study confirms that ITS sequences are appropriate for characterizing and assessing the genetic diversity of *Rhizoctonia* AG and subgroups isolates. The five *R. solani* AG isolated in this study were genetically different because

Fig. 3. One of the most parsimonious trees based on ribosomal DNA internal transcribed spacer sequences of 69 binucleate *Rhizoctonia* isolates. Maximum parsimony (MP) and maximum likelihood (ML) bootstrap support values $\geq 70\%$ from 1,000 replicates are given at the nodes. Thickened lines indicate MP and ML of 100% each. Tester strains recommended by Sharon et al. (2008) were indicated in bold. The tree was rooted to *Athelia rolfsii*.

they clustered into five distinct clades in both the MP and ML analysis. Phylogenetic analysis of the ITS region indicated that all South African AG 3-PT isolates were mostly genetically homogenous, although three small subclades were present, despite the wide variability of other biological features such as virulence and morphological characteristics.

Detailed analysis of the ITS region of AG 3-PT isolates revealed sequence variation within the ITS 1 and ITS 2 regions. The ITS 1 region showed more sequence variation than the ITS 2 region, indicating different rates of change, while 5.8S is conserved. Sequence polymorphism within the ITS region might suggest that ITS sequences are associated with different nuclei, because *R. solani* AG 3-PT isolates are multinucleate organisms. ITS sequence heterogeneity in AG 3-PT has also been previously reported in AG 3-PT and other AG (Das et al. 2014; Fiers et al. 2011; Sharon et al. 2008).

The pathogenicity results showed that isolates within the same AG had great variability in pathogenicity and virulence, which may be isolate dependent rather than AG dependent. Generally, the ability of AG 3-PT isolates to cause black scurf on potato tubers was higher than other AG, indicating a specialized nature of different AG. In pathogenicity testing, the two binucleates were able to cause stem canker on potato stems and black scurf on potato tubers. AG R was more aggressive in causing stem canker and black scurf than AG A. These results are in accordance with previous reports showing that BNR cause potato diseases on stems. Yang et al. (2014) demonstrated that AG A, AG F, AG K, and AG U were capable of causing brown lesions on potato stems in China. In the United Kingdom, an unknown BNR caused elephant hide and stem and root infection (Woodhall et al. 2011). To our knowledge, this is the first report of AG R causing black scurf on potato tubers.

This work has provided improved knowledge about *R. solani* groups and subgroups found on potato in different locations in South Africa. It has confirmed the predominance of AG 3-PT associated with potato disease in South Africa but it has also shown that AG 2-IIIB, AG 4HG-I, AG 4HG-III, and AG 5 are present and pathogenic to potato. This is the first report of BNR AG A and AG R causing stem and stolon canker and black scurf of potato in South Africa. This is also the first report of *R. solani* AG 2-IIIB and AG 4HG-I

causing stem and stolon canker of potato in South Africa. Knowledge of the AG present is important for selection of effective disease management strategies. For example, some AG are highly susceptible to certain fungicides while others are not (Campion et al. 2003; Kataria and Gisi 1999) and the host range of individual AG differs (Ogoshi 1996). 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 work is needed to evaluate efficacy of fungicides used in control programs and the pathogenicity of different AG occurring in potato cropping systems on rotation crops in South Africa.

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Table 5. Disease index for *Rhizoctonia solani* and binucleate isolates representing different anastomosis groups (AG) on potato^z

Isolate	AG, subgroup	Stem canker	Stolon canker	Black scurf
Rh20	2-IIIB	35 ef	0 a	0 a
Rh23	2-IIIB	35 ef	37 d	0 a
Rh86	2-IIIB	37 f	0 a	0 a
Rh28	3-PT	25 cde	0 a	18 c
Rh35	3-PT	35 ef	0 a	0 a
Rh36	3-PT	33 ef	15 b	41 i
Rh37	3-PT	33 ef	10 B	35 h
Rh42	3-PT	63 h	33 d	29 g
Rh44	3-PT	25 cde	33 d	23 cdef
Rh45	3-PT	44 fg	32 cd	21 cd
Rh46	3-PT	44 fg	10 b	35 h
Rh54	3-PT	44 fg	33 d	17 c
Rh102	3-PT	44 fg	33 d	23 cdef
Rh69	3-PT	44 fg	33 d	21 cdef
Rh71	3-PT	15 bc	0 a	35 h
Rh77	3-PT	15 bc	0 a	0 a
Rh89	3-PT	0 a	44 e	17 c
Rh88	4HG-I	10 ab	0 a	0 a
Rh91	4HG-I	15 bcd	25 c	0 a
Rh81	4HG-III	44 fg	0 a	0 a
Rh82	4HG-III	52 g	0 a	0 a
Rh83	4HG-III	25 cde	0 a	0 a
Rh30	5	35 ef	15 b	10 b
Rh80	A	33 ef	0 a	10 b
Rh113	R	44 fg	0 a	27 dfg

^z Means followed by the same letter within a column are not significantly different from each other according to Duncan's Multiple Range Test ($\alpha = 0.05$).

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