Anastomosis groups and pathogenicity of *Rhizoctonia solani* and binucleate *Rhizoctonia* from potatoes in South Africa

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A survey of anastomosis groups (AGs) of *Rhizoctonia* species associated with potato diseases was conducted in South Africa. A total of 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. *Rhizoctonia solani* isolates recovered belonged to AG 3-PT, AG 2-2IIIB, 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. *Rhizoctonia solani* AG 3-PT was the most predominant AG and occurred in all the potato growing regions sampled whereas the other AGs occurred in distinct locations. Different AGs 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 AGs showed differences in aggressiveness between and within AGs. Isolates of AG 2-2IIIB, 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 potatoes in South Africa using a molecular-based approach. This is the first report of *R. solani* AG 2-2IIIB and AG 4 HG-I causing stem and stolon canker and BNR AG A and AG R causing stem canker and black scurf on potatoes in South Africa.

Keywords: black scurf, ITS sequences, potato, phylogeny, pathogenicity, stem and stolon canker

Introduction

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 *Rhizoctonia,* which is a globally ubiquitous fungus (Sneh et al. 1991).

Rhizoctonia species 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* causes significant quantitative and qualitative yield losses globally (Abd-Elsalam et al. 2009; Campion et al. 2003; Das et al. 2014; Fiers et al. 2011; Tsror 2010; Woodhall et al. 2008). Marketable yield losses caused by *Rhizoctonia* spp. on potatoes have been estimated to reach 30% (Banville 1989). *Rhizoctonia* is 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 above ground symptoms of *Rhizoctonia* infection of potatoes (Banville 1989; Tsror 2010).

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

Isolates of *R. solani* differ in phenotypic and genotypic characteristics, and have been traditionally arranged in genetically related groups based on hyphal anastomosis grouping. Hitherto, 13 AGs, 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 AGs, AG A to AG U including several subgroups (Sharon et al. 2008; Tsror, 2010). BNR have been reported to be pathogenic to potatoes in Finland (Lehtonen et al. 2008), Great Britain (Woodhall et al. 2011), United States of America (Miles et al. 2013) and in 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 AGs have host preferences and are associated with specific disease symptoms. *Rhizoctonia solani* AG 3 is predominantly associated with Solanaceae crops and are subgrouped into AG 3-PT on potato, AG 3-TB on tobacco and AG 3-TM on tomato (Bartz et al. 2010; Kuninanga et al. 2000**)**. Globally, AG 3-PT has been considered as 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; Tsror 2010). In addition to *R. solani* AG 3-PT, several other AGs 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).

Delineation of *R. solani* into AGs 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 AGs 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 AGs 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 relative 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 AGs 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 AGs 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. This study therefore 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, geographic location under which the potatoes were grown was noted (Table 1).

To obtain *Rhizoctonia* isolates, infected plant material showing visible signs of *Rhizoctonia* symptoms were washed in running tap water and dried in a laminar flow cabinet for 4 h. Small pieces of infected tissue, 4 mm 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 50 mg/l of streptomycin sulphate (Sigma-Aldrich). 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* species 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 seeds were placed as bait in 90 mm petri dishes containing soil samples and incubated for 48 h at 25°C.

After incubation, bait seeds were transferred from soil to 1.5% WA amended with 50 mg/l of streptomycin, 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 sub-cultured on PDA for 7 days at 25°C. Fungal mycelia was 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, Irvine CA, USA) according to the manufacturer's protocol recommendations. The concentration and quality of extracted DNA was determined by NanoDrop UV spectrophotometry (NanoDrop Technologies).

PCR 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 template DNA, 250 µM of each dATP, dTTP, dGTP, dCTP (Bioline, London, UK), 10x NH_4 reaction buffer consisting of 160 mM $(NH_4)_2SO_4$, 670 mM Tris-HCl at pH8.8 and 100 mM KCl (Bioline), 0.25 U BIOTaq™ DNA polymerase (Bioline), 3 mM $MgCl_2$ and 0.2 µM of 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, 72°C for 45 s and a final extension of

72°C for 5 min. A 5 µl aliquot of each PCR product was separated by electrophoresis on 1.5% (w/v) of Metaphor agarose (Lonza) stained with ethidium bromide solution (0.1 mg/l) and visualized using a UV transilliuminator. 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 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 analysed 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).

Phylogenetic analysis

Consensus sequences were compared to 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; Woodhall et al. 2012; Yang et al. 2014) for the different AGs 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 PAUP 4.0* (Phylogenetic Analysis Using Parsimony* and Other Methods version 4) (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 (TBR) branch swapping,

MULPAR effective and MaxTrees set to auto-increase. The consistency (CI) and retention (RI) indices were determined for the data sets. Bootstrap analyses were performed to determine branching point confidence intervals (1000 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 (AIC), was determined with jModeltest 2.1.7 (Darriba et al. 2012; Guindon and Gascuel 2003). For the *R. solani* dataset a TPM2uf+I+G model and for the BNR dataset a TIM3+G model was used and 1000 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

Twenty five isolates belonging to different AGs: AG 3-PT (Rh28, Rh35, Rh36, Rh37, Rh42, Rh44, Rh45, Rh46, Rh54, Rh69, Rh71, Rh77, Rh89, Rh102,); AG 2-2IIIB (Rh20, Rh23, Rh86); AG 4HG-I (Rh88, Rh91); AG 4HG-III (Rh81, Rh82, Rh83); AG 5 (Rh30); and AG A (Rh80), 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 (cv. Mondial) was tested in greenhouse conditions at 25±2°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 colonised. A sprouted seed tuber, cultivar Mondial, was planted in a 5 liter pot containing sterile potting mixture of pine bark, sand and soil at 1:1:1 (w/w). Ten grams of colonized barley grains 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 complete block

design. Each replicate contained eight plants. Four plants from each replicate were destructively sampled four weeks after inoculation for stem and stolon canker disease index assessment.

Disease severity rating was done according to Woodhall et al. (2008), using a 0-4 scale where 0 = no damage or lesions present; 1 = one to several lesions less than 5 mm in size; 2 = lesions larger than 5 mm and some girdling present; 3 = larger lesions and girdling; 4 = all stems/stolons killed. *Rhizoctonia* diseases on stems and stolons were presented as disease index (DI).

Disease index was calculated by the formula: $DI= \sum [0(n_0) + 0.2(n_1) + 0.4(n_2) + 0.6(n_3) +0.8(n_4) +1(n_5)] \times 100/ (N_{total})$ where n_x = number of stems or stolons in the x rating class and N = total number of stems or stolons.

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 % of the tuber surface area covered in sclerotia; 2 = 1 to 10 % of the tuber surface area covered in sclerotia; 3 = 11 to 20 %; 4 = 21 to 50 %; 5 = ≥ 51 % tuber surface area covered in sclerotia. The disease index was calculated as: $DI= \sum [0(n_0) + 0.25(n_1) + 0.5(n_2) + 0.75(n_3) +1(n_4)] \times 100/ (N_{total})$, where n_x = number of tubers in the x rating class and N = total number of tubers in each of the category. To fulfil 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 randomised block design with three replications. Data were subjected to analysis of variance (ANOVA) and treatment means were separated by Duncan's multiple range test (α=0.05). The data was analysed using GENSTAT 14th Edition (VSN International).

Results

Collection and identification of *Rhizoctonia* isolates

A total of 131 isolates conforming to the *Rhizoctonia* species concept were obtained from ten 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 (112) were multinucleate and identified as *R. solani*, whereas 19 isolates were binucleate. Assignment of isolates to the various AGs 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 2-2IIIB, 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 2-2IIIB (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 2-2IIIB and AG 4HG-III, and most of AG A (81%) isolates were also obtained from other symptoms on tubers like 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 the all the potato growing regions sampled (Fig. 1). AG 2-2IIIB isolates were recovered from two growing regions, North West and Sandveld; while AG 4HG-III was obtained from one growing region, Limpopo. Binucleate *Rhizoctonia* 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 potatoes in South Africa using universal primers ITS1-F and ITS-4 generated a fragment of 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. Eleven polymorphic sequence types (p1 - p11) and 24 monomorphic sequence types (m1 – 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 binucleate *Rhizoctonia* 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, consistency index 0.6621 and retention index 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 2-2IIB in the MP analysis. The isolates from South Africa grouped into five distinct clades corresponding to AG 3-PT, 5, 2-2IIIB, 4HG-III and 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, consistency index 0.6211 and retention index 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 sub-clade within the AG A clade in the ML analysis, compared to 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, K, Fa, U and I. One isolate from potato (KC782951) in the USA 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 since 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 AGs isolated in this study showed that there was an inter- and intra-variation in aggressiveness of *Rhizoctonia* AGs 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 towards potatoes 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 to those from AG 3-PT isolates. AGs 2-2IIIB, 3-PT, 4HG-III, A and R were aggressive on stems causing stem canker and AG 3-PT was more aggressive on stolons causing stolon canker than any other AGs. 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.

Discussion

In this study, a survey of anastomosis groups of *R. solani* and binucleate *Rhizoctonia* 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 AGs 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 anastomosis groups 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). Besides 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 to the observation of Lehtonen et al. (2009) who postulated that AG 3-PT is much more efficient in producing sclerotia on tubers than other AGs, reflecting a highly specialized nature of AG 3-PT in infecting potato.

In this study besides AG 3-PT, members of other *R. solani* AGs 2-2IIIB, 4HG-I, 4HG-III, and 5 were retrieved from different *Rhizoctonia* 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 to be associated with black scurf on potato in France (Campion et al. 2003), UK (Woodhall et al. 2007) and South Africa (Truter and Wehner 2004).

The majority of AG 2-2IIIB isolates (88%) were found in samples from the North West growing region. The localized occurrence of anastomosis group 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 2-2IIIB 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 2-2IIIB 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 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* AGs recovered from the potato cropping system.

AG 2-1 was not detected in this study despite the fact that they it has been previously been 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 potatoes were grown in South Africa, its effect on potato was not demonstrated (Truter and Wehner 2004). The variability in the range of AGs present in potato fields in different countries could be related to selection factors that favour the occurrence of certain AGs 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. 2014; Yang et al. 2014), they were isolated from tuber blemishes in this study. The BNR isolated in this study belonged to anastomosis groups AG A and AG R. This research supports

previous observations that *Rhizoctonia* disease in potato crops could be caused by several anastomosis groups 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 anastomosis groups. This study confirms that ITS sequences are appropriate for characterizing and assessing the genetic diversity of *Rhizoctonia* anastomosis groups and subgroups isolates. The five *R. solani* AGs isolated in this study were genetically different as 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 sub-clades were present, despite the wide variability of other biological features, like virulence and morphological characteristics.

Detailed analysis of 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 as *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 AGs (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 anastomosis groups indicating a specialized nature of different AGs. 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. The 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 UK an unknown binucleate *Rhizoctonia* caused elephant hide, 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 subgroup 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 2-2IIIB, 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 2-2IIIB and AG 4HG-I causing stem and stolon canker of potato in South Africa. Knowledge of the AG(s) present is important for selection of effective disease management strategies. For example, some AGs are highly susceptible to certain fungicides whilst others are not (Campion et al. 2003; Kataria and Gisi 1999) and the host range of individual AGs differs (Sneh et al. 1996). Therefore effective implementation of *Rhizoctonia* disease control strategies on potatoes using crop rotation and fungicides should be guided by the presence of a particular AG in a particular area. Further work is therefore needed to evaluate efficacy of fungicides used in control programmes, and the pathogenicity of different AGs occurring in potato cropping systems on rotation crops in South Africa.

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

Isolate[a]	AG	Symptom of origin	GenBank Accession number	Potato growing region	Potato variety	Preceding crop	ITS type[b]
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	KJ777586	KwaZulu-	Potato	Potato	m2

		rhizosphere soil		Natal	rhizosphere Soil		
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
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	2-2IIIB	Stem canker	KJ777569	North West	Mondial	Sugarbeet	nd
Rh21	2-2IIIB	Stem canker	KJ777570	North West	Mondial	Sugarbeet	nd
Rh22	2-2IIIB	Stem canker	KJ777571	North West	Mondial	Maize	nd
Rh23	2-2IIIB	Corky spots	KJ777572	North West	Mondial	Maize	nd
Rh31	2-2IIIB	Elephant hide	KJ777580	North West	Mondial	Sugarbeet	nd
Rh97	2-2IIIB	Black scurf	KJ777643	Sandveld	BP1	Unknown	nd
Rh99	2-2IIIB	Stem canker	KJ777645	North West	BP1	Maize	nd
Rh86	2-2IIIB	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
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

[a]Isolates with the same numerical value originated from the same tuber but from different blemish types

[b]m types contain monomorphic sequences and p types contain polymorphic sequences

nd=not determined

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

Isolate no	Anastomosis group	Host plant or substrate	Geographic origin	GenBank accession no
		Binucleate *Rhizoctonia*		
1Fuk-600[a]	A	Rose	Japan	AB196663
4Oit-800[a]	A	Rose	Japan	AB196661
AH-1[a]	A	Peanut	Japan	AB196639
C-538[a]	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[a]	A	Soil	United States of America	DQ102417
AH-2	Fa (F)[b]	Potato	China	KF176561

HuB-6	Fa (F)[b]	Potato	China	KF176581
HuN-1-1	Fa (F)[b]	Potato	China	KF176582
PS-17[a]	Fa	Pea	Japan	AB219144
SX-2	Fa (F)[b]	Potato	China	KF176606
Str10[a]	Fa	Strawberry	Israel	DQ102434
Str36[a]	Fa	Strawberry	Israel	DQ102435
AH-6[a]	Fb	Peanut	Japan	AB196645
Bn38[a]	Fb	Soybean	United States of America	AF354081
FKO2-28[a]	Fb	Soil	Japan	AB219145
55D21[a]	I	Sugar beet	Japan	AB290023
AV-2[a]	I	*Artemisia* sp.	Japan	AB196650
HuN-4-1	I	Potato	China	KF176586
Im2[a]	I	Strawberry	United States of America	DQ102444
R92	I (unknown)[b]	Potato	Finland	DQ913035
206	K (A)[b]	Potato	United States of America	KC782951
56D17[a]	K	Sugar beet	Japan	AB286932
AC-1[a]	K	Onion	Japan	AB122145
GS-19	K	Potato	China	KF176568
HeB-16	K	Potato	China	KF176571
NM-19-1	K	Potato	China	KF176597
SH-10[a]	K	Soil	Japan	AB196652
SX-7	K	Potato	China	KF176609
Bn-37[a]	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[a]	R	Ginger	China	AB286942
Xb-1-3[a]	R	*Betula* sp.	China	DQ885784
HuN-3	U	Potato	China	KF176585
MWR-20[a]	U	Rose	Japan	AB196664
MWR-22[a]	U	Rose	Japan	AB196666
MWR-24[a]	U	Rose	Japan	AB196665
cc43	Unknown	Potato	United Kingdom	FR828480
		Rhizoctonia solani		
DB13MAG05	2-2IIIB	*Rebutia perplexa*	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	*Arachis hypogaea*	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

[a]Binucleate *Rhizoctonia* tester isolates recommended by Sharon et al. (2008).

[b]Original assigned anastomosis group indicated in brackets.

Table 3 Incidence of individual AGs of *Rhizoctonia solani* and binucleate *Rhizoctonia* isolates collected from each symptom

Symptom of origin	AG3-PT	AG 2-2IIIB	AG 4HG-I	AG 4HG-III	AG5	AGA	AGR	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 5 Disease index for *Rhizoctonia solani* and binucleate isolates representing different anastomosis groups on potato

Isolate	AG/Subgroup	Stem canker		Stolon Canker		Black Scurf	
Rh20	2-2IIIB	35	ef	0	a	0	a
Rh23	2-2IIIB	35	ef	37	d	0	a
Rh86	2-2IIIB	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

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

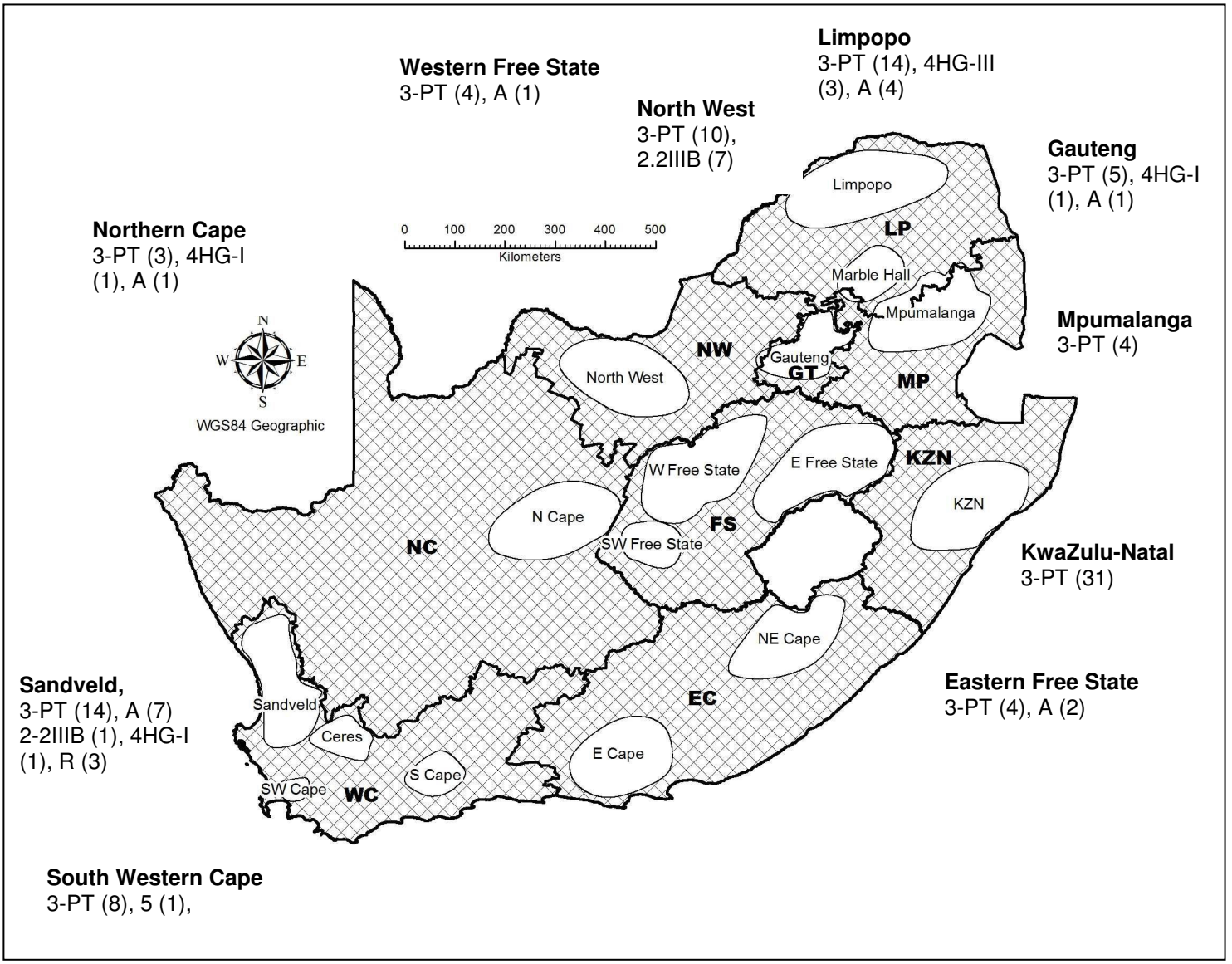
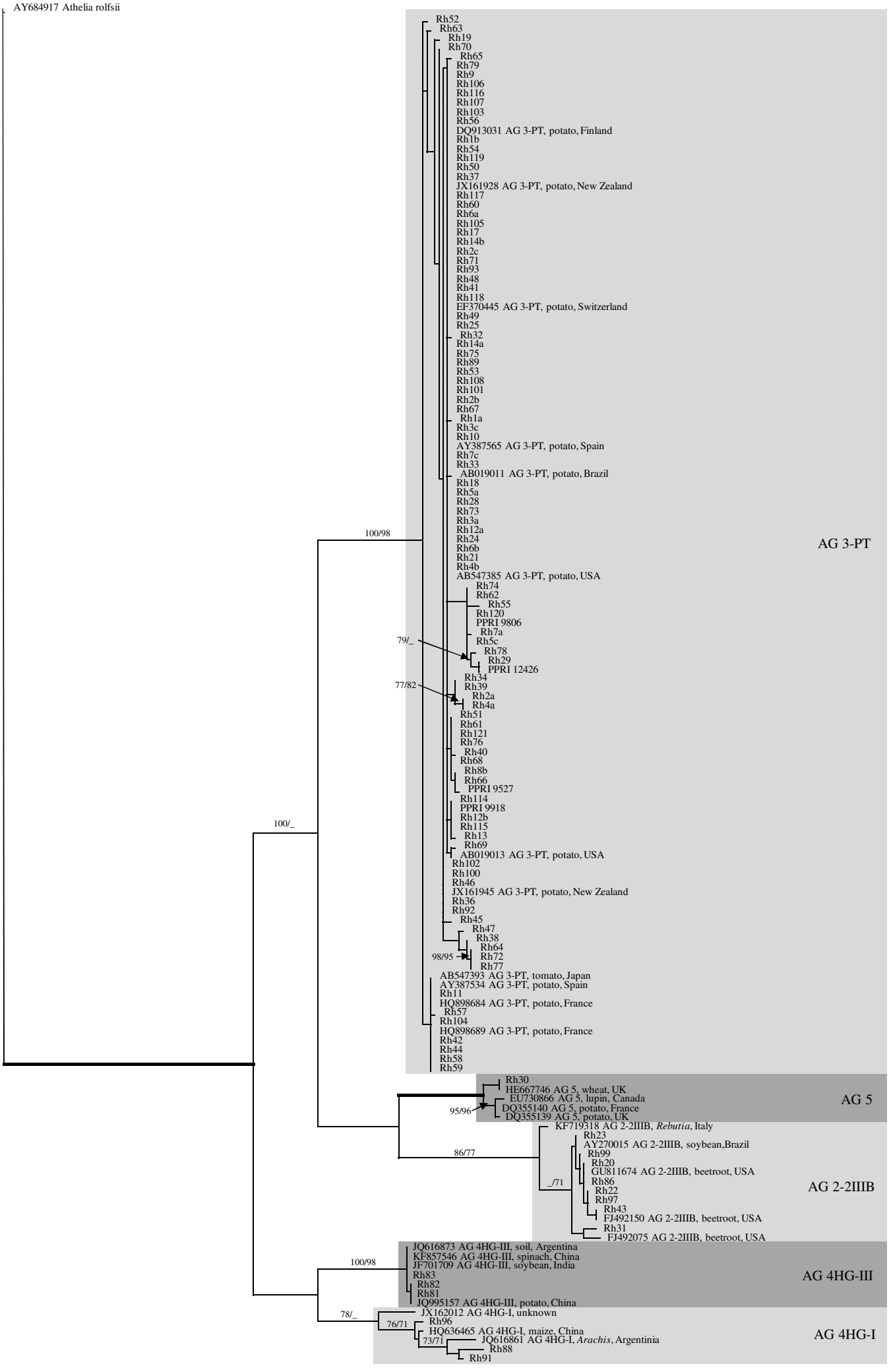


Figure 1 Map showing sampling sites and distribution of various Rhizoctonia anastomosis groups in major potato growing regions in South Africa.


AY684917 Athelia rolfsii
Rh52
Rh63
Rh19
Rh70
Rh65
Rh79
Rh9
Rh106
Rh116
Rh107
Rh103
Rh56
DQ913031 AG 3-PT, potato, Finland
Rh1b
Rh54
Rh119
Rh50
Rh37
JX161928 AG 3-PT, potato, New Zealand
Rh117
Rh60
Rh6a
Rh105
Rh17
Rh14b
Rh2c
Rh71
Rh93
Rh48
Rh41
Rh118
EF370445 AG 3-PT, potato, Switzerland
Rh49
Rh25
Rh32
Rh14a
Rh75
Rh89
Rh53
Rh108
Rh101
Rh2b
Rh67
Rh1a
Rh3c
Rh10
AY387565 AG 3-PT, potato, Spain
Rh7c
Rh33
AB019011 AG 3-PT, potato, Brazil
Rh18
Rh5a
Rh28
Rh73
Rh3a
Rh12a
Rh24
Rh6b
Rh21
Rh4b
AB547385 AG 3-PT, potato, USA
Rh74
Rh62
Rh55
Rh120
PPRI 9806
Rh7a
Rh5c
Rh78
Rh29
PPRI 12426
Rh34
Rh39
Rh2a
Rh4a
Rh51
Rh61
Rh121
Rh76
Rh40
Rh68
Rh8b
Rh66
PPRI 9527
Rh114
PPRI 9918
Rh12b
Rh115
Rh13
Rh69
AB019013 AG 3-PT, potato, USA
Rh102
Rh100
Rh46
JX161945 AG 3-PT, potato, New Zealand
Rh36
Rh92
Rh45
Rh47
Rh38
Rh64
Rh72
Rh77
AB547393 AG 3-PT, tomato, Japan
AY387534 AG 3-PT, potato, Spain
Rh11
HQ898684 AG 3-PT, potato, France
Rh57
Rh104
HQ898689 AG 3-PT, potato, France
Rh42
Rh44
Rh58
Rh59
AG 3-PT
100/98
79/_
77/82
98/95
100/_
Rh30
HE667746 AG 5, wheat, UK
EU730866 AG 5, lupin, Canada
DQ355140 AG 5, potato, France
DQ355139 AG 5, potato, UK
AG 5
95/96
KF719318 AG 2-2IIIB, *Rebutia*, Italy
Rh23
AY270015 AG 2-2IIIB, soybean,Brazil
Rh99
Rh20
GU811674 AG 2-2IIIB, beetroot, USA
Rh86
Rh22
Rh97
Rh43
FJ492150 AG 2-2IIIB, beetroot, USA
Rh31
FJ492075 AG 2-2IIIB, beetroot, USA
AG 2-2IIIB
86/77
_/71
JQ616873 AG 4HG-III, soil, Argentina
KF857546 AG 4HG-III, spinach, China
JF701709 AG 4HG-III, soybean, India
Rh83
Rh82
Rh81
JQ995157 AG 4HG-III, potato, China
AG 4HG-III
100/98
JX162012 AG 4HG-I, unknown
Rh96
HQ636465 AG 4HG-I, maize, China
JQ616861 AG 4HG-I, *Arachis*, Argentinia
Rh88
Rh91
AG 4HG-I
78/_
76/71
73/71
10

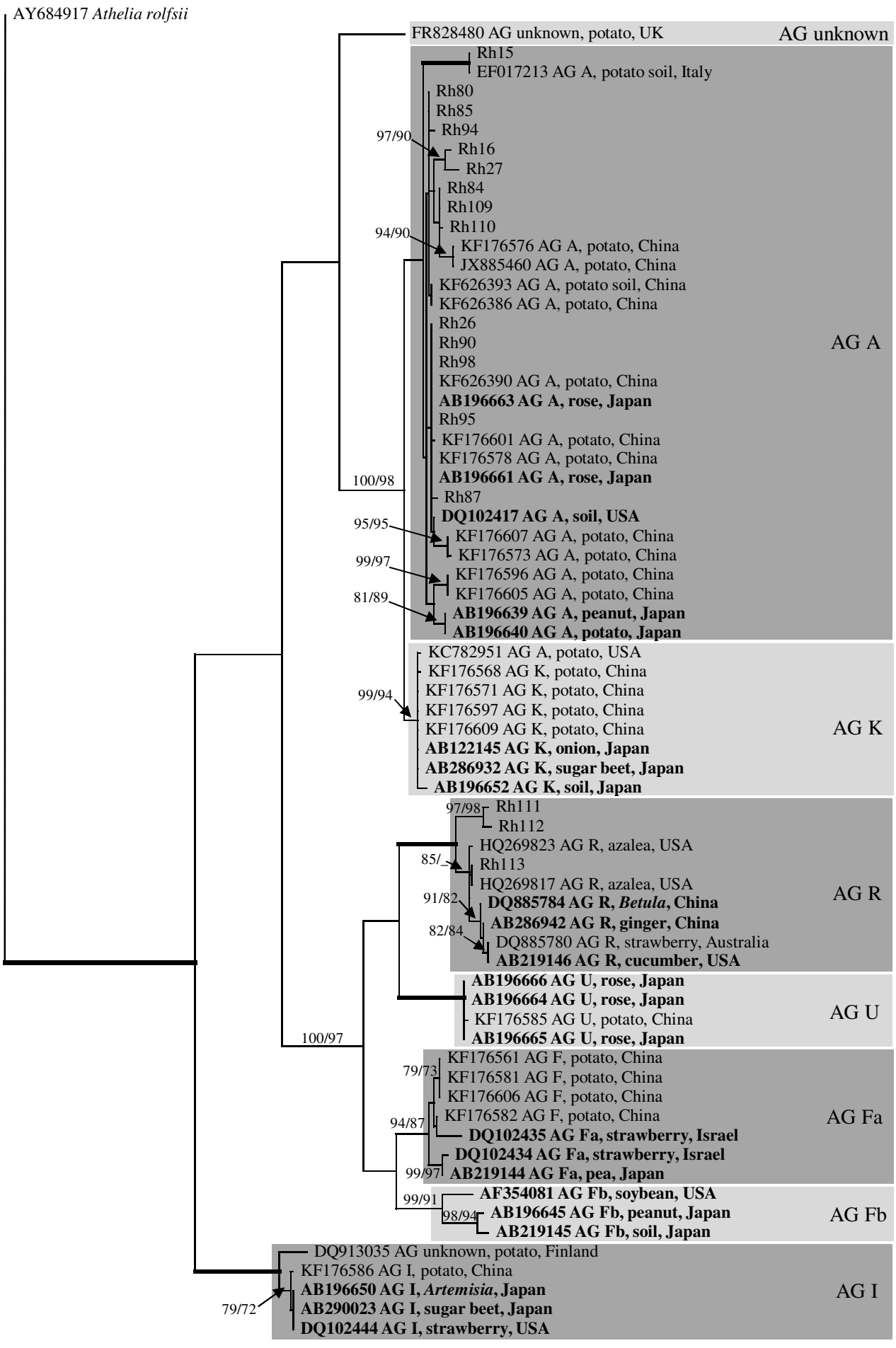


Figure 2 One of the most parsimonious trees based on rDNA ITS sequences of 146 Rhizoctonia solani isolates. The maximum parsimony (MP) and maximum likelihood (ML) bootstrap support values ≥70% from 1000 replicates are given at the nodes. Thickened lines indicate MP and ML of 100% each. The tree was rooted to Athelia rolfsii.

eXtra 1 Symptoms on greenhouse grown potato plants inoculated with different AGs of *Rhizoctonia* (**A**) Black scurf caused by AG 3-PT isolate Rh 37 (**B**) Stem canker caused by AG 4HGIII isolate Rh83 (**C**) Stolon canker caused by AG 3-PT Rh 44

Figure 3 One of the most parsimonious trees based on rDNA ITS sequences of 69 binucleate Rhizoctonia isolates. The maximum parsimony (MP) and maximum likelihood (ML) bootstrap support values ≥70% from 1000 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.