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Anastomosis Groups of *Rhizoctonia solani* and Binucleate *Rhizoctonia* Associated with Potatoes in Idaho

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Abstract

A survey of the relative incidence of anastomosis groups (AGs) of *Rhizoctonia* spp. associated with potato disease was conducted in Idaho, the leading potato producing state in the U.S.A. In total, 169 isolates of *Rhizoctonia solani* and seven binucleate *Rhizoctonia* (BNR) isolates were recovered from diseased potato plants. The AG of each isolate was determined through real-time PCR assays for AG 3-PT and phylogenetic analysis of the internal transcribed spacer region of ribosomal DNA. AG 3-PT was the predominant AG, accounting for 85% of isolates recovered, followed by AG 2-1 (5.7%) and AG 4 HG-II (4.5%). Two different subsets of AG 2-1 isolates were recovered (subset 2 and 3). Three isolates each of AG A and AG K were recovered, as well as one isolate each of AG 5 and AG W. An experiment carried out under greenhouse conditions with representative isolates of the different AGs recovered from Idaho potatoes showed differences in aggressiveness between AGs to potato stems, with AG 3-PT being the most aggressive followed by an isolate of AG 2-1 (subset 3). The three BNR isolates representative of AG A, AG K, and AG W appeared to be less aggressive to potato stems than the *R. solani* isolates except for the AG 2-1 (subset 2) isolate. This is the first comprehensive study of the relative incidences of *Rhizoctonia* species associated with Idaho potatoes and the first study to report the presence of BNR AG W outside of China.

Keywords: black scurf, Rhizoctonia potato disease, *Solanum tuberosum*, stem canker, *Thanatephorus cucumeris*

Rhizoctonia potato disease is an important disease of potatoes. Infection can result in marketable yield losses of up to 30% (Banville 1989). Quality losses are typically associated with the development of sclerotia on the surface of progeny tubers (black scurf), although other tuber blemish symptoms such as elephant hide can occur (Fiers et al. 2010; Muzhinji et al. 2014). Rhizoctonia potato disease can also cause mishappen tubers (Tsror 2010) as well as affecting the specific gravity and chip color quality of processed tubers (Otrysko and Banville 1992). Quantitative losses can occur from the infection of stems, stolons, and roots, which affects tuber size and number (Tsror 2010). The main causal agent is the multinucleate fungus *Rhizoctonia solani*, although some binucleate *Rhizoctonia* (BNR) fungi have also been reported to infect potatoes (Muzhinji et al. 2015; Yang et al. 2015).

Isolates of *R. solani* can be assigned to one of 13 known anastomosis groups (AGs) (Carling et al. 2002), whereas BNR are assigned to AG A through W (Yang et al. 2015). Traditionally, AGs were assigned by observing hyphal fusion with known tester isolates. However, internal transcribed spacer (ITS) sequence analysis is now the most appropriate method for the classification of *Rhizoctonia* (Sharon et al. 2006, 2008), with a range of conventional PCR and real-time PCR assays also available (Budge et al. 2009; Woodhall et al. 2013). These molecular methods can often determine the AG present with considerably less ambiguity than AG determination through observation of hyphal fusion with tester isolates.

In potatoes, individual AGs have been associated with different types of disease. For example, AG 3-PT has been associated with stem canker and black scurf, whereas AG 8 causes almost exclusively root disease (Woodhall et al. 2008). Although AG 3-PT is typically the predominant AG on potatoes worldwide (Tsror 2010), there have been instances where other AGs have predominated, like AG 4 in parts of Peru (Anguiz and Martin 1989), or where the majority of isolates are not AG 3-PT, as in Brazil (Inokuti et al. 2019). In addition, there have been instances where the other AGs that were encountered were particularly aggressive on potatoes, such as with certain AG 2-1 isolates encountered in Great Britain (Woodhall et al. 2007) and with all New Zealand AG 2-1 isolates (Das et al. 2014). Considerable diversity exists within AG 2-1 and several studies have attempted to further differentiate isolates. Carling et al. (2002) assigned AG 2-1 isolates into one of three subsets based on ITS sequence. Subset 1 included the Japanese AG 2-1 culture type II and Dutch AG 2t isolates, subset 2 contained Alaskan and Australian AG 2-1 isolates, and subset 3 included Italian tobacco isolates (designated as 'AG 2-1/Nt', and previously designated as 'AG 2-Nt'). Misawa et al. (2018) analyzed additional AG 2-1 sequences and confirmed that the Carling et al. (2002) subsets represented distinct clades of AG 2-1 and added two further clades: the HK clade (for Hokkaido) consisting of isolates from Japan, Belgium, and the Netherlands, and the UK clade consisting of potato isolates from the UK and Finland. Isolates of the UK clade were determined to have a shorter intergenic spacer 1 sequence compared with subset 3 isolates (Woodhall et al. 2007) and also appeared to be less aggressive to potato stems than subset 3 isolates (Woodhall et al. 2008).

The AG present is an important consideration for *Rhizoctonia* diseases. Differences exist between AGs in host range, disease severity, optimal temperature growth, sclerotia production, and fungicide sensitivity (Kataria and Gisi 1999; Tsror 2010). Therefore, knowledge of the occurrence of individual AGs in a specific area and their relative aggressiveness on potato is essential for predicting disease development and correct disease management. Idaho is the leading potato producing state in the U.S., but no data exists on the

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incidence of individual AGs on the potato crop in the state. Therefore, the aim of this study was to characterize isolates of *Rhizoctonia* from potatoes grown in Idaho and to determine the relative incidence of individual AGs using a previously published diagnostic assay for AG 3-PT (Woodhall et al. 2013) in conjunction with ITS sequencing. The relative aggressiveness on potato stems of isolates representative of each AG or subset was also determined.

Materials and Methods

Isolation and characterization

Between June 2018 and November 2020, potato samples displaying symptoms of Rhizoctonia potato disease were received at the diagnostic lab at Parma Research and Extension Center, Idaho, U.S.A. Samples were either those submitted for diagnosis from growers and consultants, tuber samples submitted for seed tuber health assessment, or from field scouting. Seed tuber health assessment samples consisted of 200 tubers and all tubers were visually examined for the presence of Rhizoctonia disease. In total, 65 separate fields were scouted for symptoms of Rhizoctonia potato disease between the months of June and September each year. Samples were washed and infected material was excised (approximately 5 mm^3) and surface sanitized in sodium hypochlorite (2%) for approximately 1 min. After rinsing twice in sterile water, the material was placed on water agar amended with penicillin G (0.2 g/liter) and streptomycin sulfate (0.8 g/liter). After 3 to 5 days, colonies of *Rhizoctonia* were identified based on the presence of septate hyphae sometimes with right-angle branching. Hyphal tips from representative colonies were transferred onto potato dextrose agar (PDA) amended with penicillin G (0.2 g/liter) and streptomycin sulfate (0.8 g/liter). The colonies were grown on PDA for 2 to 3 weeks, after which isolates were sorted based on macromorphology and a single isolate representative of each macromorphological type for each symptom in each sample was selected. For long term storage, isolates were kept on PDA slants at room temperature.

DNA extraction and polymerase chain reaction

DNA was extracted from isolates using a Wizard Magnetic DNA Purification System for Food (Promega) in conjunction with a Kingfisher ML magnetic particle processor (Thermo Fisher Scientific, Waltham, MA) following the manufacturer's recommended protocols. Real-time PCR was carried out in 96-well plates using a QuantStudio 3 system (Applied Biosystems, Waltham, MA) with a 20 μl reaction volume. Two microliters of template DNA (approximately 10 ng in each reaction) was used in all reactions. For SYBR green real-time PCR, PowerUp Master (Applied Biosystems) was used, while Environmental Master Mix 2.0 (Applied Biosystems) was used for TaqMan. Master Mix made up half of the reaction volume. Primers and probes were supplied by Eurofins Genomics (Louisville, KY). Primers were added to a final concentration of 300 nM and probes, when required for TaqMan, to 100 nM with the remaining volume made up with water. Cycling conditions for SYBR green were 50°C for 2 min, 95°C for 2 min, and 40 cycles of 95°C for 15 s and 60°C for 1 min. For TaqMan, the first 95°C step was for 10 min instead of 2 min. The Ct (cycle threshold) value for each reaction was assessed using the default threshold on the analysis software. The primers and probes used in the study are given in Table 1. Two sets of published assays were verified for specificity for AG 3-PT using ITS sequenced isolates available in the culture collection at Parma. Primers and probe sequences were not modified from the original published studies although the tetramethylrhodamine (TAMRA) 3′ modification for probe AG3-PT_P was substituted with a Black Hole Quencher (BHQ1).

DNA sequencing and phylogenetic analysis

The real-time PCR assay from Woodhall et al. (2013) was initially used to determine if AG 3-PT was present. Isolates with an observed Ct of 40, as well as representative AG 3-PT isolates, were subjected to ITS sequencing. PCR SuperMix (Invitrogen, Waltham, MA) was used with 200 mM of primers ITS4 and ITS5 with 2 μl of template DNA. Thermal cycling was done on a SimpliAmp (Applied Biosystems) PCR machine with initial denaturation at 94°C for 75 s, followed by 35 cycles of 94°C for 15 s, annealing temperature at 58°C for 15 s and 72°C for 45 s, the final cycle having an extra 4 min 15 s at 72°C. PCR products were purified using a Qiagen PCR prep kit and verified using gel electrophoresis before using DNA sequencing in both directions by Eurofins Genomics. ClustalW was used to align the sequences in MEGA 11 (Tamura et al. 2021). DNA sequences from known reference isolates used in previous studies (Misawa et al. 2018; Muzhinji et al. 2015; Yang et al. 2015) taken from GenBank were also used in the phylogenetic analysis. Datasets for BNR and *R. solani* were analyzed separately by the maximum-likelihood (ML) method using MEGA and a methodology adapted from Misawa et al. (2018). The general time reversible model (Nei and Kumar 2000) with a discrete gamma distribution model was used to model evolutionary rate differences among sites. Complete deletion was set as gap/missing data treatment. The strength of the internal branches of the resulting tree was tested with boot-strap analysis using 1,000 replications. The resulting tree was visualized with MEGA. A sequence of AG G (GenBank AB196646) was used as the outgroup for *R. solani* sequences, while an AG 5 sequence (GenBank DQ355140) was used for the BNR analysis.

Pathogenicity of representative isolates to potato stems

Representative isolates of each AG or subset of AG 2-1 found in this study on potato were tested under glasshouse conditions of approximately 21°C with a 16 h/8 h light/dark cycle and watered as required. All isolates were from Idaho potatoes except for AG 5, which was isolated from an Idaho wheat stem as the potato AG 5 isolate was not available at the time of planting. Seed tuber pieces (cv. Russet Burbank) with single sprouts approximately 25 mm in length were planted in individual pots (127 mm wide, 114 mm deep) containing potting mix (Fertiloam ultimate potting mix, Austin, TX) at approximately 65 mm depth. A single 10 mm^2 PDA plug fully colonized with the appropriate isolate was placed next to the sprout and then potting mix was added to ensure the whole sprout was covered. A sterile PDA plug was used as a noninoculated control treatment. Each treatment was replicated 10 times. After 21 days, plants were removed from pots, washed, and assessed for stem canker disease using the key described in Carling and Leiner (1990) as follows 0 = no damage or lesions present; 1 = minor damage, one to several lesions less than 5 mm in size; 2 = moderate damage,

Table 1. Sequences of the primers and probes used for real-time PCR in this study

Assay	Description	Primer/probe name	Sequence (5′-3′)[z]	Length (bp)
Woodhall et al. (2013)	Forward	AG3-PT_F	ATGAAGAGTTTGGTTGTAGCTGGTCT	26
	Reverse	AG3-PT_R	TATTACAAWAAATAACAAAT AAATTCCCCAA	31
	FAM-BHQ probe	AG3-PT_P	CCCTCTTTCATCCCACACACACCTG	25
Salamone and Okubara (2020)	Forward	AG3-WA-F3	GAACCCTTCTGTCTACTCAACTC	23
	Reverse	AG3-WA-R2	CATGCTCCAAGGAATACCAAG	21

[z] Underlined bases denote locked nucleic acid (LNA) bases, W denotes ambiguous coding for A or T.

lesions larger than 5 mm and some girdling present; 3 = major damage, large lesions and girdling or death present on most stems; 4 = all stems killed. The experiment was repeated once. From three symptomatic stems in each experiment, isolations were attempted from up to three symptomatic plants where available, and AG identity confirmed on the resulting isolates as described above. All statistical analyses were performed using the R statistical package (R Core Team 2017). Data analysis from both experiments was performed using the Tukey HSD function, which submitted the data as simple ANOVA and then generated the Tukey test comparisons, $P < 0.05$.

Results

Verification of real-time PCR assay specificity

To determine the assay to be used in this study, the AG 3-specific assay of Salamone and Okubara (2020) was compared against the Woodhall et al. (2013) assay with specificity for AG 3-PT. In this study, the Woodhall et al. (2013) primers or primer and probe combination in a SYBR green assay or TaqMan assay, respectively, was specific for AG 3-PT when tested with DNA from isolates from AG 2-1, AG 2-2, AG 3-PT, AG 4 HG-II, and AG 5, while the Salamone and Okubara (2020) assay detected DNA from isolates of AG 2-1, AG 2-2, and AG 3-PT (Table 2). The Woodhall et al. (2013) assay was therefore used for AG 3-PT identification for the remainder of the study.

Isolation and characterization

In total, 347 *Rhizoctonia* isolates were recovered from 18 samples submitted for diagnosis, 70 seed tuber samples, and 54 samples from potato fields. Of those, 176 isolates were selected for further characterization after duplicate isolates for each sample representative of the same AG/symptom type were removed. A total of 169 isolates of *R. solani* and seven isolates of BNR were recovered. Among the 169 *R. solani* isolates, 150 were determined to belong to AG 3-PT, while 10 were identified as AG 2-1, eight as AG 4 HG-II, and a single isolate was AG 5. For BNR, three isolates each of AG A and AG K were found as well as a single isolate of AG W. Anastomosis group 3-PT was isolated from all symptom categories used in this study (Table 3). At least one representative isolate for each of the AGs were recovered from stem lesions except for AG A, which was only recovered from elephant hide. Most isolates were from east Idaho, followed by southwest Idaho (Table 4). Only four different AGs were recovered from east Idaho despite a larger sample number, and five different AGs were recovered each from south central and southwest Idaho. A complete isolate list with AG, origin, and GenBank accession number is given in the supplementary data file.

DNA sequencing and phylogenetic analysis

Sequencing of the ITS region was conducted with DNA originating from all 26 isolates that tested negative for AG 3-PT, as well as DNA from eight representative AG 3-PT isolates. From the phylogenetic analysis, all isolates of AG 3-PT, AG 4 HG-II, and AG 5 grouped with sequences from the appropriate reference isolates (Fig. 1). All but one Idaho isolate of AG 2-1 grouped with AG 2-1 subset 3 sequences. One isolate (P2) clustered with the AG 2-1 subset 2 reference sequences. When seven isolates from Idaho as well as 17 reference sequences from AG A, K, W, G, L, and O were used to construct the maximum likelihood tree for BNR, three Idaho isolates grouped with AG A, three with AG K, and one with AG W (Fig. 2).

Pathogenicity of representative *Rhizoctonia* isolates to potato stems

The isolate of AG 3-PT appeared to be most aggressive to potato stems (Table 5), although this was not significantly different from the AG 2-1 subset 3 isolate in both experiments and the AG 4 HG-II isolate in one experiment only. The AG 5 isolate also caused consistent stem canker in both experiments, while AG A and AG W caused mild stem canker with smaller lesions typically observed. The AG 2-1 subset 2 isolate caused mild stem canker in one experiment only, while no stem lesions were observed in the noninoculated control plots or those inoculated with AG K. Except for plants inoculated with AG K and noninoculated controls, the AG that was used to inoculate the stem was successfully reisolated from symptomatic stems.

Discussion

In this study, the relative incidence of individual AGs of *R. solani* and BNR isolates associated with potato diseases in Idaho was determined. Isolates were shown to belong to four AGs of *R. solani*: AG 3-PT, AG 2-1, AG 4 HG-II, and AG 5, and three BNR AGs: AG A, AG K, and AG W. AG 3-PT was the predominant group found, accounting for 85% of isolates recovered. This is consistent with many previous studies worldwide where typically AG 3-PT comprises between 73 and 100% of isolates recovered (Tsror 2010). One of the reasons why AG 3-PT is so common in potatoes may be its ability to form greater numbers of sclerotia on tubers (Lehtonen et al. 2009; Woodhall et al. 2008), and although both seed- and soil-borne inoculum can initiate disease, the importance of seed-bone inoculum has previously been demonstrated in Idaho (Duellman et al. 2021).

Table 2. Cycle threshold values (Ct) generated with assays from Salamone and Okubara (2020) and Woodhall et al. (2013) with DNA originating from isolates representing *Rhizoctonia solani* AG 2-1, AG 2-2, AG 3-PT, AG 4 HG-II and AG 5[z]

Isolate #	AG	GenBank accession	Host	SYBR Green (Salamone and Okubara 2020)	SYBR Green (Woodhall et al. 2013)	TaqMan (Woodhall et al. 2013)
P119	2-1	MW462178	Potato	–	–	–
P54	2-1	MW462187	Potato	37.7	–	–
P11	2-1	MW462177	Potato	38.0	–	–
P60	2-1	MW462188	Potato	–	–	–
C174	2-1	MW462173	Wheat	–	–	–
DB72	2-2	MW462174	Bean	20.2	–	–
ON3	2-2	MT672318	Onion	17.9	–	–
P4	3-PT	MW462184	Potato	19.0	26.4	25.1
P25	3-PT	MW462180	Potato	16.7	21.4	21.0
P29	3-PT	MW462181	Potato	17.1	22.0	21.5
P36	3-PT	MW462182	Potato	17.1	22.0	20.2
P37	3-PT	MW462183	Potato	16.3	21.6	19.6
P40	3-PT	MW462185	Potato	16.0	21.3	19.2
P48	3-PT	MW462186	Potato	14.5	19.5	17.8
P108	3-PT	MW462176	Potato	15.2	19.6	18.4
P126	4 HG-II	MW462179	Potato	–	–	–
C81	5	MW462175	Wheat	–	–	–

[z] The dash symbol (–) indicates the assay did not detect DNA from the isolate.

In addition to AG 3-PT, isolates of AG 2-1 and AG 4 HG-II were also recovered from black scurf. The presence of AG 2-1 has also been observed in previous studies in Europe (Campion et al. 2003; Chand and Logan 1983; Lehtonen et al. 2009; Woodhall et al. 2007), Alaska (Carling and Leiner 1986), and New Zealand (Das et al. 2014), although none of these studies found AG 4 isolates associated with potatoes. While the study from Alaska found AG 2-1 on potatoes, other studies in North America did not. In Mexico, AG 3 and AG 4 were present (Virgen-Calleros et al. 2000), in Alberta, AG 3, AG 4, and AG 5 were all present (Bains and Bisht 1995), and in Maine, AG 3 and AG 5 were present (Bandy et al. 1988). In this study, AG 4 HG-II accounted for eight isolates and was recovered from tubers with black scurf as well as stem and root disease symptoms. AG 5 accounted for a single isolate causing stem canker. AG 4 HG-II has previously been reported to cause black scurf in Cyprus (Kanetis et al. 2016). Both AG 4 HG-II and AG 5 have previously been reported on cereal crops (Pizolotto et al. 2020; Woodhall et al. 2012b) that are grown in rotation with potatoes in Idaho; as a result, it was expected to encounter these AGs. AG 4 HG-II is also found on sugar beet in Idaho (Strausbaugh et al. 2011), a crop also often grown in rotation with potatoes.

AG 2-2 was not found in this study, which is surprising as it was previously reported causing potato disease in Idaho (Woodhall et al. 2012a) and has also been reported on sugar beet (Strausbaugh et al. 2011) and onion (Brown et al. 2021) in Idaho. AG 2-2 has also been found on South African potatoes, although it only accounted for eight of 131 isolates (Muzhinji et al. 2015), and a single AG 2-2 isolate was also found on potatoes in Turkey (Özer and Bayraktar 2015). Thus, it is likely that AG 2-2 is only present at low levels in the Idaho potato crop.

BNR isolates were also recovered, although they only accounted for seven of the 176 isolates (3.9%). This included AG A, AG K, and for the first time outside of China, AG W. In China, AG W has been reported causing disease in potatoes and sugar beet (Yang et al. 2015; Zhao et al. 2019). In this study, AG W caused similar symptoms to those reported by Yang et al. (2015) both when observed in the field and when inoculated onto stems in the greenhouse. AG W was successfully reisolated from these symptomatic stems, thereby confirming Koch's postulates. AG A was recovered from elephant hide symptoms three times in this study. In South Africa, it was found more frequently (12.2%) and was also predominantly associated with elephant hide symptoms (Muzhinji et al. 2015). Both AG A and AG K were found causing potato stem canker in China along with AG F, AG I, and AG U (Yang et al. 2014), with the BNR isolates causing significantly lower levels of stem disease compared with an isolate of AG 3. This is similar to the findings reported here except the Chinese isolates of AG K were more aggressive to potato plants than AG A.

In this study, a real-time PCR assay was used to identify isolates belonging to the predominant potato group, AG 3-PT. Real-time

Table 3. Anastomosis group of *Rhizoctonia solani* and binucleate *Rhizoctonia* isolates from Idaho potatoes collected from 2018 to 2020 by symptom

Symptom	AG 2-1	AG 3-PT	AG 4 HG-II	AG 5	AG A	AG K	AG W	Total
Stem canker	3	36	5	1		2	1	48
Stolon canker		11						11
Root lesion		2	1					3
Black scurf	6	91	2					99
Elephant hide	1	10			3	1		15
Total	10	150	8	1	3	3	1	176

Table 4. Anastomosis group of *Rhizoctonia solani* and binucleate *Rhizoctonia* isolates from Idaho potatoes collected from 2018 to 2020 by location

Origin	AG 2-1	AG 3-PT	AG 4 HG-II	AG 5	AG A	AG K	AG W	Total
Southwest	6	59	4		1		1	71
South Central		22	2	1	2	2		29
East	4	69	2			1		76
Total	10	150	8	1	3	3	1	176

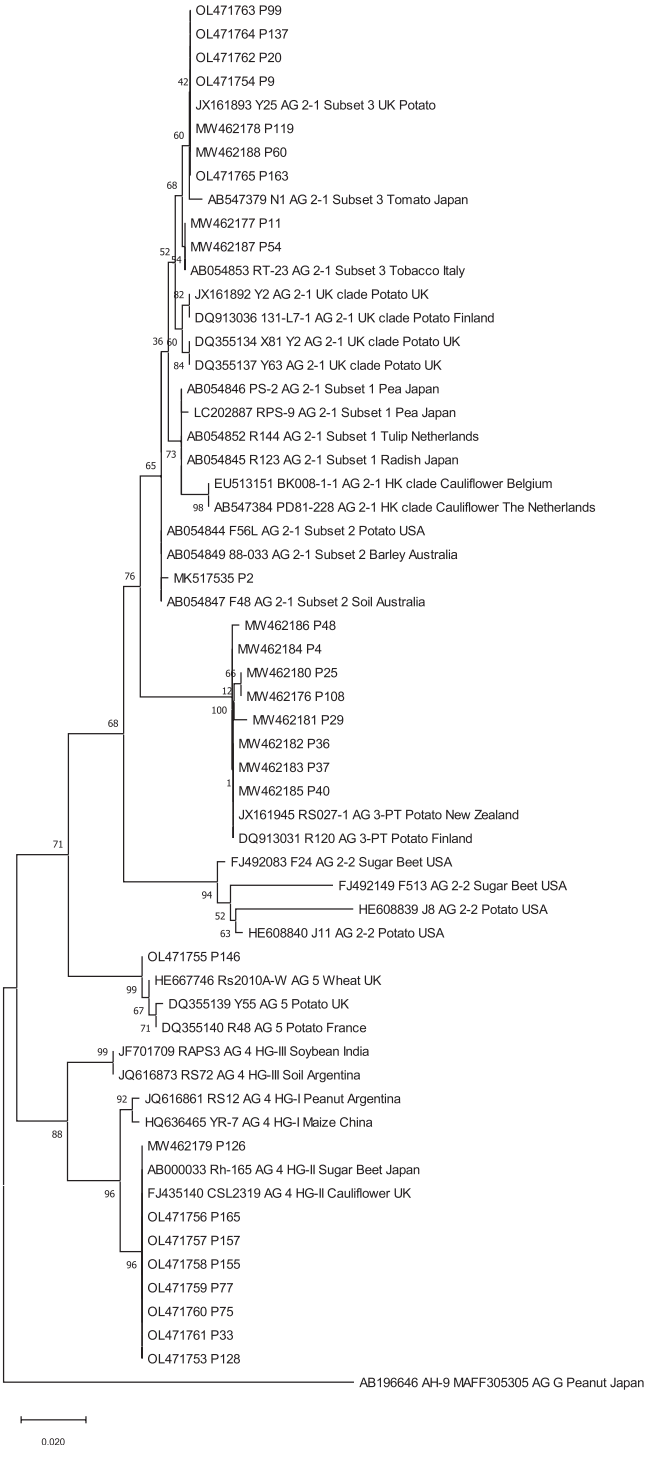

Fig. 1. Maximum likelihood tree based on rDNA-ITS sequence data for 58 isolates of *Rhizoctonia solani* of which 27 isolates were from this study. The sequence of binucleate *Rhizoctonia* AG G isolate AH-9 was used as the outgroup. AG, host, and origin are given for reference isolates while isolates from this study are designated with a P number. Numbers on branches represent percentage bootstrap values obtained from 1,000 replications. The scale bar corresponds to 0.02 substitutions per nucleotide position. Subsets or clades from Misawa et al. (2018) are given for reference AG 2-1 isolates.

PCR, like sequencing of the ITS region, avoids many of the ambiguities that can arise when an AG is identified through the observation of hyphal fusion with known tester isolates. However, it is essential that appropriate validation data are available for each assay and that the performance of the assay is verified in the lab where it will be deployed prior to use. In the current study, we compared an AG 3 assay from Salamone and Okubara (2020) with the AG 3-PT subgroup specific assay from Woodhall et al. (2013). Salamone and Okubara (2020) stated that the Woodhall et al. (2013) assay did not detect DNA from all Pacific Northwest isolates of AG 3 as under their PCR conditions, it failed to amplify DNA of one AG 3 isolate from Washington State. However, all 150 isolates of AG 3-PT from this study were successfully amplified with real-time PCR when used with the TaqMan probe. Salamone and Okubara (2020), when evaluating the Woodhall et al. (2013) assay, did not use the TaqMan probe, which could explain the differences. Previously, assays were reported to have been compromised by the lack of adherence to published protocols (Yasuhara-Bell et al. 2019). Under the reaction conditions used here, the Salamone and Okubara (2020) assay also detected DNA from AG 2-1 and AG 2-2, with particularly robust detection of AG 2-2 DNA. This highlights the need for assay verification, especially where specificity is required.

Although real-time PCR allowed for rapid and cost-effective screening of isolates in this study, ITS sequencing provided additional information on the isolates collected. The phylogenetic analysis in this study showed that at least two subgroups of AG 2-1 were present in Idaho potatoes. Most AG 2-1 isolates in this study grouped with isolates of subset 3, while one grouped with subset 2. Isolates from subset 3 have previously been shown to be aggressive to potato stems in the UK and New Zealand (Das et al. 2014), while subset 2 isolates from Alaska were less aggressive (Carling and Leiner 1986). These prior works are consistent with the pathogenicity testing in this study, where the subset 2 isolate was less aggressive, although only one isolate of each subset was evaluated. Further work with additional AG 2-1 isolates from other hosts is required to investigate the aggressiveness of individual subgroups of AG 2-1 to specific hosts and to understand the genetic determinants that influence aggressiveness.

This work demonstrates that AG 3-PT is the predominant AG in Idaho potatoes, which is consistent with similar studies from other

Table 5. Mean stem disease index based on a scale of 0 to 4 for potatoes inoculated with *Rhizoctonia* isolates representing 8 different groups with noninoculated control in the glasshouse

		Key[z]	
AG	**Isolate**	**Experiment A**	**Experiment B**
Noninoculated		0.0 c	0.0 d
AG 2-1 (subset 2)	P2	0.0 c	0.3 cd
AG 2-1 (subset 3)	P54	2.3 ab	1.9 ab
AG 3-PT	P37	2.9 a	2.7 a
AG 4 HG-II	P128	2.2 ab	0.9 c
AG 5	C184	1.7 b	1.8 b
AG A	P129	0.4 c	0.1 cd
AG K	P156	0.0 c	0.0 d
AG W	P150	0.2 c	0.5 cd
CV (%)		122.23	121.62

[z] Index of Carling and Leiner (1990). Means with the same letter within a column are not significantly different from one another (Tukey's test, $P < 0.05$).

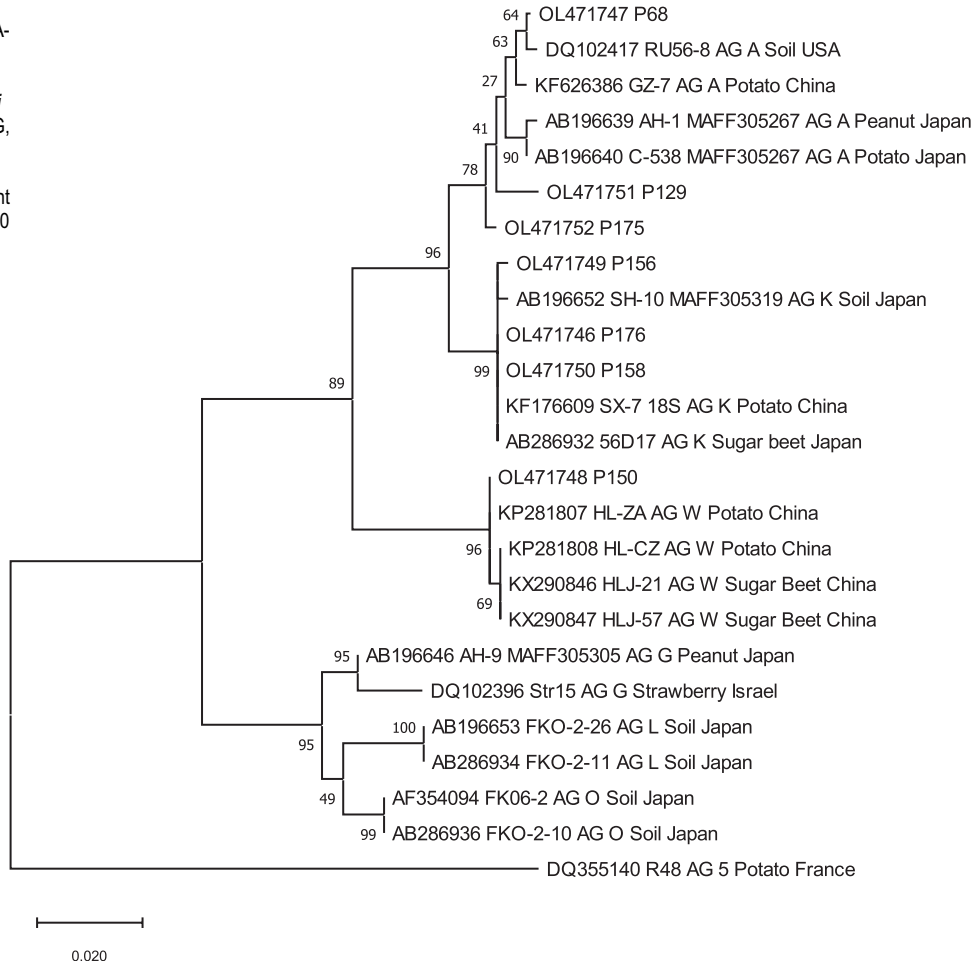

Fig. 2. Maximum likelihood tree based on rDNA-ITS sequence data for 24 isolates of binucleate *Rhizoctonia* species of which seven isolates were from this study. The sequence of *R. solani* AG 5 isolate R48 was used as the outgroup. AG, host, and origin are given for reference isolates while isolates from this study are designated with a P number. Numbers on branches represent percentage bootstrap values obtained from 1,000 replications. The scale bar corresponds to 0.02 substitutions per nucleotide position.

locations (Tsror 2010). It also shows that several other AGs are present in Idaho potatoes including AG 2-1, AG 4 HG-II, AG 5, AG A, AG K, and AG W. Knowledge of the AG present in an area is important for the selection of the most appropriate disease management strategy. This includes fungicide selection as some AGs are highly sensitive to certain fungicides while others are not (Campion et al. 2003; Kataria and Gisi 1999; Muzhinji et al. 2018) and the choice of crop rotation since host range and aggressiveness to potato can differ between individual AGs, subgroups, and for AG 2-1, even between isolate subsets (Woodhall et al. 2008).

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