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PCR With Specific Primers for Detection of *Rhizoctonia solani* Anastomosis Groups Reveals Lack of Specificity

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ABSTRACT

Several specific primers based on the internal transcribed spacer (ITS) region of rDNA have been used to discriminate anastomosis groups (AGs) and subgroups in *Rhizoctonia solani*, but the efficacy of these primers was not evaluated considering several known AGs. This study aimed to evaluate the efficacy of seven PCR-specific primers for the detection of four AGs and subgroups of *R. solani* (AG-1 IA, AG-1 IB, AG-2-1, AG-3 PT, AG-3 TB, AG-4 HGI, and AG-4 HGII). Thirteen isolates of *R. solani* and seven isolates of binucleate *Rhizoctonia* belonging to different AGs and subgroups were used in the detection assays and were amplified and sequenced using ITS1 and ITS4 universal primers to confirm the previous identification of AGs and the viability of the DNA samples. In addition, three isolates of unrelated fungal species (*Fusarium oxysporum, Macrophomina phaseolina,* and *Sclerotium rolfsii*) were tested simultaneously with each primer set above as a negative control. All primers tested nonspecifically amplified other AGs, and most of the primers produced bands for unrelated fungal species. Therefore, the exclusive use of these primers under the PCR conditions should be avoided due to the lack of accuracy in the results.

The soil-borne fungus *Rhizoctonia* occurs worldwide in agricultural and non-agricultural fields, living as plant pathogens, symbionts, or saprophytes (Gónzalez et al. 2016). The study of *Rhizoctonia* is largely associated with its economic importance as a pathogen on more than 600 species of plants (USDA 2025), causing symptoms such as damping off, root rot, stem cankers, crown rot, and blights (Ogoshi 1996).

The genus *Rhizoctonia* comprises a complex of genetically distinct species, and the classification is based on the cell's nuclear condition (multi-, bi-, or uninucleate) and the ability of hyphae to anastomose with tester isolates of designated anastomosis groups (AGs) (Sneh et al. 1991). *Rhizoctonia solani* (teleomorf *Thanatephorus cucumeris*) is a multinucleate and the most important species within the genus *Rhizoctonia* (Sun et al. 2024). Currently, there are 13 AGs in *R. solani* (AG-1 to AG-13) and numerous subgroups (Arakawa and Inagaki 2014; Gónzalez et al. 2016; Sharon et al. 2006; Sun et al. 2024).

The classical methodology for AG-grouping based on hyphal interaction has proven to be a questionable and unreliable criterion for subgroups. On the other hand, the introduction of polymerase chain reaction (PCR)-based tools provided the accurate identification of isolates for AGs and their subgroups (Sharon et al. 2006).

Sequencing of the internal transcribed spacer (ITS) region of ribosomal DNA (rDNA) seems to be an appropriate method for the genetic differentiation of AGs and their subgroups (Sharon et al. 2008), and these differences are applied to the design of molecular markers for distinguishing those groups (Arakawa and Inagaki 2014). Several specific primers based on the ITS region have been designed to discriminate AGs and their subgroups in the *R. solani* (Arakawa and Inagaki 2014). These specific primers have been used in several research works (Budge et al. 2009; Farhaoui et al. 2024; Hannukkala et al. 2016; Matsumoto 2002; Misawa et al. 2017; Mora-Umaña et al. 2013; Okubara et al. 2008; Su et al. 2024; Wang et al. 2023), but the efficacy of these primers was not evaluated considering several known AGs. In this context, the aim of this study was to evaluate the efficacy of seven PCR-specific primers for the detection of four *R. solani* AGs (AG-1 IA, AG-1 IB, AG-2-1, AG-3 PT, AG-3 TB, AG-4 HGI, and AG-4 HGII).

Thirteen isolates of *R. solani* and seven isolates of binucleate *Rhizoctonia* belonging to different AGs and subgroups were used in this study (Table 1). Isolates of *Fusarium oxysporum*, *Macrophomina phaseolina*, and *Sclerotium rolfsii* (Table S1), previously characterised by DNA sequencing, were used as a negative control.

The isolates of *Rhizoctonia, F. oxysporum*, *M. phaseolina*, and *S. rolfsii* were grown on a cellophane disc placed on potato-dextrose-agar (PDA) (Acumedia, Lansing, USA) in a Petri dish at 25°C. After 4 days, the genomic DNA was obtained using the Wizard Genomic DNA Purification Kit (Thermo Fisher Scientific, Waltham, USA) following the manufacturer's instructions. Amplification reactions using the specific primer pairs for *Rhizoctonia* AGs detection were carried out using 1 μL of genomic DNA (25 ng/μL), 6.25 μL of GoTaq Master Mix (Promega), 0.5 μL of each primer, and 4.25 μL of ultrapure water, and the PCR conditions specified in each reference for each primer are cited in Table 2. All primers were supplied by Thermo Fisher Scientific. Unrelated fungal species (*F. oxysporum*, *M. phaseolina*, and *S. rolfsii*) were also tested with each primer pair. A negative control with 1 μL of nuclease-free water was performed for all sets of primers during PCR reactions. The PCR amplification products were separated by electrophoresis in 1% agarose gels in 1.0× tris-acetate acid EDTA (TAE) buffer, stained with GelRed (Biotium Inc., Hayward, USA) and visualised under UV light and photographed. The tests were repeated twice.

For the confirmation of the identity of the *Rhizoctonia* isolates and the DNA samples viability, the ITS-rDNA region was amplified using the universal primers ITS1 and ITS4 (White et al. 1990) using the same reagents and concentrations

TABLE 1 | *Rhizoctonia solani* (Rs) and binucleate *Rhizoctonia* (BNR) isolates used in evaluation of specific primers for detection of anastomosis groups (AG).

Isolate code	Reference	AG[a]	Host plant or substrate	Geographic origin	GenBank accession no.
CS-KA	Rs	1 IA	Rice	Japan	MT591306
SHIBA-2	Rs	1 IB	Potato	Japan	MT591307
C-96	Rs	2–2 IIIB	Mat rush	Japan	MT591308
RI-64	Rs	2–2 IV	Beet	Japan	MT591309
TE2-4	Rs	2 BI	Soil	Japan	MT591310
CMM-1806	Rs	3 PT	Potato	Brazil	MT591311
AH-1	Rs	4 HGI	Peanut	Japan	MT591312
MMBF45/11	Rs	4 HGII	Beet	United States	MT591313
CMM-2989	Rs	4 HGIII	Cowpea	Brazil	MT591314
CU-8904	Rs	5	Squash	China	MT591315
HO-1556	Rs	7	Soil	Japan	MT591316
Sq R1	Rs	9	Soil	United States	MT591317
RH-31	Rs	11	Soybean	United States	MT591318
CMM-2473	BNR	A	Potato	Brazil	MT591319
MMBF38/11	BNR	Ba	Soybean	Brazil	MT591320
MMBF25/11	BNR	Bb	Soybean	United States	MT591321
MMBF40/11	BNR	F	Soybean	Brazil	MT591322
MMBF39/11	BNR	G	Soybean	Brazil	MT591323
MMBF35/11	BNR	P	Soybean	Brazil	MT591324
CMM-1315	BNR	R	Potato	Brazil	MT591325

[a]Anastomosis group.

TABLE 2 | Primer sets for detection of anastomosis groups (AG) of *Rhizoctonia solani* used in this study, with annealing temperature (AT) and expected amplicon size (AS).

AG	Primer sequence (forward/reverse)	AT[a] (°C)	AS[b] (bp)	References
AG-1 IA	CCTTAATTTGGCAGGAGGG	58	540	Kuninaga (2003)
	GACTATTAGAAGCGGTTCA			
AG-1 IB	ACACTAGAGTAGGTGGTATCA	53	324	Grosch et al. (2007)
	AGCGTGCTAACATAGTCACTC			
AG-2-1	CAAAGGCAAT(A/G)GGTTATTGGAC	60	480	Carling et al. (2002)
	CCTGATTTGAGATCAGATCATAAAG			
AG-3 PT	GTTTGGTTGTAGCTGGTCT	65	470	Kuninaga et al. (2000)
	CTGAGATCCAGCTAATAC			
AG-3TB	GTTTGGTTGTAGCTGGCCC	65	470	Kuninaga et al. (2000)
	CTGAGATCCAGCTAATGT			
AG-4 HGI	GGACCTACTCTC(C/T)TTGG	55	420	Kuninaga (2003)
	ACAGGGTGTCCTCAGCGA			
AG-4 HGII	GGACCTTCTACTCCCCCT	55	420	Kuninaga (2003)
	ACAGGGTGTCCTCAGCGA			

[a]Annealing temperature.
[b]Amplicon size.

mentioned above. The PCR conditions consisted of an initial denaturation at 94°C for 5 min followed by 30 cycles of denaturation at 94°C for 30 s, annealing at 52°C for 40 s and elongation at 72°C for 40 s each; and a final extension at 72°C for 5 min. PCR products were purified and sequenced at Macrogen (Seoul, South Korea). Each fragment was sequenced in both directions with the same primers used for amplification.

The obtained sequences were assembled using the SeqMan Pro version 8.1.2 software (DNASTAR Inc., Madison, USA). Reference ITS sequences available in GenBank for each AG were retrieved for the construction of a phylogenetic tree (Table S2). Sequences were aligned using the ClustalW algorithm (Thompson et al. 1997), and phylogenetic analyses were done separately for multinucleate and binucleate *Rhizoctonia* datasets using MEGA 7.0.14 (Kumar et al. 2016). Maximum Likelihood with the General Time Reversible nucleotide substitution model was used to construct the phylogenetic trees. Bootstrapping was performed with 1000 replications of data. The ITS sequence of *Athelia rolfsii* (FSR-052) (GenBank Accession no. AY684917) was used as an outgroup. Sequences generated in this study were deposited in GenBank.

The results of the phylogenetic analysis of the ITS region of *Rhizoctonia* isolates allowed the identification of 20 AGs (Table 1), including 13 of *R. solani* (Figure S1) and seven of binucleate *Rhizoctonia* (Figure S2), and confirmed the AGs classification previously performed for some isolates (Carling et al. 2002; Kuninaga et al. 1997; Matsumoto 2002; Strausbaugh et al. 2011).

The primer sets produced the expected product for the representative isolates from AG-1 IA and AG-1 IB, with an evident band of 540 bp and 324 bp, respectively. However, nonspecific annealing was visualised in other AGs for this primer set (Table 3 and Figures S3 and S4). For primer set AG-2-1, although we do not have isolates belonging to this subgroup, PCR products of the expected size were visualised in the AG-9, AG-F, and AG-P isolates, as well as the presence of amplicon for the AG-4 HGI isolate (Table 3 and Figure S5). The AG-3 PT primer set amplified DNA from the AG-3 PT isolate, but with amplicon above 500 bp, different from that indicated by Kuninaga et al. (2000). In addition, it was observed that the presence of multiple non-specific bands, with different sizes for all subgroups, except for the AG-1 IA subgroup. In the AG 3-TB primer set, PCR products of the expected size (470 bp) were not visualised, since we do not have isolates from this AG. However, amplicons were evident for AG-4 HGI, AG-F, and AG-G isolates (Table 3 and Figures S6 and S7). In the case of the AG-4 HGI primer set, a band fragment of the expected size (420 bp) was produced for the AG-4 HGI isolate, but multiple bands of different sizes were detected for other subgroups. The AG-4 HGII primer set produced a single band of the expected size (420 bp) for the representative isolate of this group, but a band of expected size was also visualised for isolates of AG-2-2 IIIB and AG-2-2 IV, as well as multiple bands for other subgroups (Table 3 and Figures S8 and S9). The pair of primers AG-1 IA, AG-3 PT, AG-3TB, AG-4 HGI, and AG-4 HGII produced bands for *F. oxysporum*, *M. phaseolina*, and *S. rolfsii* used as negative control during the PCR reactions. No PCR product was observed in the control (water).

Although specific primers have been previously used for the detection of *Rhizoctonia* AGs and subgroups (Budge et al. 2009; Farhaoui et al. 2024; Hannukkala et al. 2016; Matsumoto 2002; Misawa et al. 2017; Mora-Umaña et al. 2013; Okubara et al. 2008; Su et al. 2024; Wang et al. 2023), their specificity was often not evaluated, or assessments were based on a limited number of AGs (Carling et al. 2002; Kuninaga et al. 2000; Salazar

TABLE 3 | Amplification results of anastomosis groups (AG) of *Rhizoctonia solani* and binucleate *Rhizoctonia* isolates and other fungi using specific primers.

	Amplification results using specific primers[a]						
AG	**AG-1 IA**	**AG-1 IB**	**AG-2-1**	**AG-3 PT**	**AG-3 TB**	**AG-4 HGI**	**AG-4 HGII**
Rhizoctonia solani							
AG-1 IA	+	–	–	–	*	*	*
AG-1 IB	–	+	–	*	*	*	*
AG-2-2 IIIB	*	–	–	*	*	*	+
AG-2-2 IV	–	–	–	*	*	*	+
AG-2 BI	–	–	–	*	*	*	*
AG-3 PT	–	–	–	*	*	*	*
AG-4 HGI	–	–	*	*	*	+	*
AG-4 HGII	–	–	–	*	*	*	+
AG-4 HGIII	–	–	*	*	*	*	*
AG-5	–	–	–	*	*	*	*
AG-7	–	*	*	*	*	*	*
AG-9	–	–	+	*	*	*	*
AG-11	*	–	–	*	*	*	*
Binucleate *Rhizoctonia*							
AG-A	–	*	–	*	*	*	*
AG-Ba	–	–	–	*	*	*	*
AG-Bb	–	–	*	*	*	*	*
AG-Fa	–	*	+	*	*	*	*
AG-G	–	–	–	*	*	*	*
AG-P	–	–	+	*	*	*	*
AG-R	–	–	–	*	*	*	*
Negative control	–	–	–	–	–	–	–
Fusarium oxysporum	*	–	–	*	*	*	*
Sclerotium rolfsii	*	–	–	*	*	*	*
Macrophomina phaseolina	*	–	–	*	*	*	*

[a]According to Table 2. (+) Presence of a single evident band with expected size; (*) presence of band with size not expected or nonspecific amplification; (–) absence of band.

et al. 2000). The present study assessed the efficacy of previously designed and published specific primers using a comprehensive panel of *R. solani* and binucleate *Rhizoctonia* isolates representing 20 distinct AGs.

The amplification observed across multiple *Rhizoctonia* isolates representing distinct AGs indicates that the primers designed for the detection of AG-1 IA, AG-1 IB, AG-2-1, AG-3 PT, AG-3 TB, AG-4 HGI, and AG-4 HGII exhibit considerable nonspecificity. Although these primers were developed based on sequence divergence within the ITS region, assumed to be unique to each AG (Carling et al. 2002; Matsumoto 2002), our findings reveal notable cross-reactivity among isolates from genetically unrelated AGs, including both multinucleate and binucleate *Rhizoctonia* forms. This lack of specificity aligns with earlier findings by Matsumoto (2002), who reported that certain specific primers developed from ITS regions exhibited cross-reactivity among different AGs of *R. solani*, leading to ambiguous or false-positive identifications.

The observed nonspecificity of some PCR primers designed for *Rhizoctonia* AG detection can be attributed, at least in part, to the extensive genetic diversity both within and between species in the *Rhizoctonia* complex. Numerous studies have demonstrated significant intraspecific variation among isolates within the same AG, as well as interspecific similarities among distinct AGs (Misawa et al. 2017; Sneh et al. 1991), which can compromise primer specificity. These overlapping molecular signatures

often stem from the evolutionary complexity of the genus, which comprises multinucleate and binucleate forms with a high degree of genomic plasticity (Nizamani et al. 2025).

Further complicating the development of truly specific molecular markers is the possibility that currently recognised AGs may not represent the full spectrum of *Rhizoctonia* diversity. Several recent studies have reported isolates with atypical morphological, pathological, or molecular characteristics that do not conform to any known AG, suggesting the presence of novel or cryptic AGs in natural environments (Alaei et al. 2017; Dong et al. 2017; Gaino et al. 2010; Moliszewska et al. 2023; Yang et al. 2015). This underlines the dynamic and potentially expanding nature of the AG classification system.

Additionally, the capacity of *Rhizoctonia* to form heterokaryons further contributes to genetic variability in field populations. These heterokaryotic interactions, along with potential sexual recombination events in the teleomorphic stage (e.g., *T. cucumeris*), can result in novel genotypic combinations, even within a single AG (Misawa et al. 2017). Such genomic rearrangement can produce isolates with mosaic ITS regions or other sequence polymorphisms that hinder detection or lead to misidentification in primer-based assays.

In conclusion, the present study revealed that other AGs could be nonspecifically amplified using the primer pairs AG-1 IA, AG-1 IB, AG-2-1, AG-3 PT, AG-3 TB, AG-4 HGI, and AG-4 HGII. This finding indicated that the exclusive use of these primers under the PCR conditions used in this study should be avoided due to the lack of accuracy in the results.

Molecular markers such as the large subunit of ribosomal DNA (LSU), β-tubulin (BT), and the second largest subunit of RNA polymerase II (RPB2) have demonstrated strong phylogenetic resolution and are highly effective for distinguishing among *Rhizoctonia* AGs (González et al. 2001, 2006; Gónzalez et al. 2016; Sharon et al. 2006). Consequently, the development of new specific primers targeting these or other highly variable gene regions is essential for accurate AG detection.

Author Contributions

Project conceptualisation: Sami Jorge Michereff and Kamila Câmara Correia. Methodology: Alexandre Reis Machado, Cristina Maria de Souza-Motta, Juan Manuel Tovar-Pedraza, Ailton Reis, Kamila Câmara Correia and Sami Jorge Michereff. Investigation: Lucas Correia Santana Amancio and Kamila Câmara Correia. Resources: Sami Jorge Michereff. Data analysis: Alexandre Reis Machado, Kamila Câmara Correia and Sami Jorge Michereff. Preparation of the draft manuscript: Lucas Correia Santana Amancio. Review and editing manuscript: Alexandre Reis Machado, Juan Manuel Tovar-Pedraza, Ailton Reis, Kamila Câmara Correia and Sami Jorge Michereff. All authors read and approved the final manuscript.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Peer Review

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Figure S1:** Maximum likelihood tree generated from sequence analysis of the ITS region dataset of reference isolates *Rhizoctonia solani*. Bootstrap support (> 70%) values for maximum likelihood are shown the nodes. The tree was rooted to *Athelia*. **Figure S2:** Maximum likelihood tree generated from sequence analysis of the ITS region dataset of reference isolates binucleate *Rhizoctonia*. Bootstrap support (> 70%) values for maximum likelihood are shown the nodes. The tree was rooted to *Athelia*. **Figure S3:** Amplification of genomic DNA using the primer sets of AG-1 IA; M = Molecular marker (1-Kb ladder marker); 1 = CS-KA (AG1-IA), 2 = SHIBA-2 (AG1-IB), 3 = C-96 (AG-2-2 IIIB), 4 = RI-64 (AG-2-2 IV), 5 = TE2-4 (AG-2 BI), 6 = CMM-1806 (AG-3 PT), 7 = AH-1 (AG-4 HGI), 8 = MMBF45/11 (AG-4 HGII), 9 = CMM-2989 (AG-4 HGIII), 10 = CU-8904 (AG-5), 11 = HO-1556 (AG-7), 12 = Sq R1 (AG-9), 13 = RH-31 (AG-11), 14 = CMM-1806 (AG-A), 15 = AH-1 (AG-Ba), 16 = MMBF25/11 (AG-Bb), 17 = MMBF40/11(AG-F), 18 = MMBF39/10 (AG-G), 19 = MMBF35/10 (AG-P), 20 = CMM-1315 (AG-R), 21 = CMM-13 (*Fusarium oxysporum*), 22 = CMM-3065 (*Sclerotium rolfsii*), 23 = CMM-4047 (*Macrophomina phaseolina*) and 24 = water (ddH_2O). **Figure S4:** Amplification of genomic DNA using the primer sets of AG-1 IB; M = Molecular marker (1-Kb ladder marker); 1 = CS-KA (AG1-IA), 2 = SHIBA-2 (AG1-IB), 3 = C-96 (AG-2-2 IIIB), 4 = RI-64 (AG-2-2 IV), 5 = TE2-4 (AG-2 BI), 6 = CMM-1806 (AG-3 PT), 7 = AH-1 (AG-4 HGI), 8 = MMBF45/11

(AG-4 HGII), 9 = CMM-2989 (AG-4 HGIII), 10 = CU-8904 (AG-5), 11 = HO-1556 (AG-7), 12 = Sq R1 (AG-9), 13 = RH-31 (AG-11), 14 = CMM-1806 (AG-A), 15 = AH-1 (AG-Ba), 16 = MMBF25/11 (AG-Bb), 17 = MMBF40/11(AG-F), 18 = MMBF39/10 (AG-G), 19 = MMBF35/10 (AG-P), 20 = CMM-1315 (AG-R), 21 = CMM-13 (*Fusarium oxysporum*), 22 = CMM-3065 (*Sclerotium rolfsii*), 23 = CMM-4047 (*Macrophomina phaseolina*) and 24 = water (ddH_2O). **Figure S5:** Amplification of genomic DNA using the primer set of AG-2-1; M = Molecular marker (1-Kb ladder marker); 1 = CS-KA (AG1-IA), 2 = SHIBA-2 (AG1-IB), 3 = C-96 (AG-2-2 IIIB), 4 = RI-64 (AG-2-2 IV), 5 = TE2-4 (AG-2 BI), 6 = CMM-1806 (AG-3 PT), 7 = AH-1 (AG-4 HGI), 8 = MMBF45/11 (AG-4 HGII), 9 = CMM-2989 (AG-4 HGIII), 10 = CU-8904 (AG-5), 11 = HO-1556 (AG-7), 12 = Sq R1 (AG-9), 13 = RH-31 (AG-11), 14 = CMM-1806 (AG-A), 15 = AH-1 (AG-Ba), 16 = MMBF25/11 (AG-Bb), 17 = MMBF40/11(AG-F), 18 = MMBF39/10 (AG-G), 19 = MMBF35/10 (AG-P), 20 = CMM-1315 (AG-R), 21 = CMM-13 (*Fusarium oxysporum*), 22 = CMM-3065 (*Sclerotium rolfsii*), 23 = CMM-4047 (*Macrophomina phaseolina*) and 24 = water (ddH_2O). **Figure S6:** Amplification of genomic DNA using the primer sets of AG-3 PT; M = Molecular marker (1-Kb ladder marker); 1 = CS-KA (AG1-IA), 2 = SHIBA-2 (AG1-IB), 3 = C-96 (AG-2-2 IIIB), 4 = RI-64 (AG-2-2 IV), 5 = TE2-4 (AG-2 BI), 6 = CMM-1806 (AG-3 PT), 7 = AH-1 (AG-4 HGI), 8 = MMBF45/11 (AG-4 HGII), 9 = CMM-2989 (AG-4 HGIII), 10 = CU-8904 (AG-5), 11 = HO-1556 (AG-7), 12 = Sq R1 (AG-9), 13 = RH-31 (AG-11), 14 = CMM-1806 (AG-A), 15 = AH-1 (AG-Ba), 16 = MMBF25/11 (AG-Bb), 17 = MMBF40/11(AG-F), 18 = MMBF39/10 (AG-G), 19 = MMBF35/10 (AG-P), 20 = CMM-1315 (AG-R), 21 = CMM-13 (*Fusarium oxysporum*), 22 = CMM-3065 (*Sclerotium rolfsii*), 23 = CMM-4047 (*Macrophomina phaseolina*) and 24 = water (ddH_2O). **Figure S7:** Amplification of genomic DNA using the primer sets of AG-3 AG-3 TB (b); M = Molecular marker (1-Kb ladder marker); 1 = CS-KA (AG1-IA), 2 = SHIBA-2 (AG1-IB), 3 = C-96 (AG-2-2 IIIB), 4 = RI-64 (AG-2-2 IV), 5 = TE2-4 (AG-2 BI), 6 = CMM-1806 (AG-3 PT), 7 = AH-1 (AG-4 HGI), 8 = MMBF45/11 (AG-4 HGII), 9 = CMM-2989 (AG-4 HGIII), 10 = CU-8904 (AG-5), 11 = HO-1556 (AG-7), 12 = Sq R1 (AG-9), 13 = RH-31 (AG-11), 14 = CMM-1806 (AG-A), 15 = AH-1 (AG-Ba), 16 = MMBF25/11 (AG-Bb), 17 = MMBF40/11(AG-F), 18 = MMBF39/10 (AG-G), 19 = MMBF35/10 (AG-P), 20 = CMM-1315 (AG-R), 21 = CMM-13 (*Fusarium oxysporum*), 22 = CMM-3065 (*Sclerotium rolfsii*), 23 = CMM-4047 (*Macrophomina phaseolina*) and 24 = water (ddH_2O). **Figure S8:** Amplification of genomic DNA using the primer sets of AG-4 HGI; M = Molecular marker (1-Kb ladder marker); 1 = CS-KA (AG1-IA), 2 = SHIBA-2 (AG1-IB), 3 = C-96 (AG-2-2 IIIB), 4 = RI-64 (AG-2-2 IV), 5 = TE2-4 (AG-2 BI), 6 = CMM-1806 (AG-3 PT), 7 = AH-1 (AG-4 HGI), 8 = MMBF45/11 (AG-4 HGII), 9 = CMM-2989 (AG-4 HGIII), 10 = CU-8904 (AG-5), 11 = HO-1556 (AG-7), 12 = Sq R1 (AG-9), 13 = RH-31 (AG-11), 14 = CMM-1806 (AG-A), 15 = AH-1 (AG-Ba), 16 = MMBF25/11 (AG-Bb), 17 = MMBF40/11(AG-F), 18 = MMBF39/10 (AG-G), 19 = MMBF35/10 (AG-P), 20 = CMM-1315 (AG-R), 21 = CMM-13 (*Fusarium oxysporum*), 22 = CMM-3065 (*Sclerotium rolfsii*), 23 = CMM-4047 (*Macrophomina phaseolina*) and 24 = water (ddH_2O). **Figure S9:** Amplification of genomic DNA using the primer sets of AG-4 HGII; M = Molecular marker (1-Kb ladder marker); 1 = CS-KA (AG1-IA), 2 = SHIBA-2 (AG1-IB), 3 = C-96 (AG-2-2 IIIB), 4 = RI-64 (AG-2-2 IV), 5 = TE2-4 (AG-2 BI), 6 = CMM-1806 (AG-3 PT), 7 = AH-1 (AG-4 HGI), 8 = MMBF45/11 (AG-4 HGII), 9 = CMM-2989 (AG-4 HGIII), 10 = CU-8904 (AG-5), 11 = HO-1556 (AG-7), 12 = Sq R1 (AG-9), 13 = RH-31 (AG-11), 14 = CMM-1806 (AG-A), 15 = AH-1 (AG-Ba), 16 = MMBF25/11 (AG-Bb), 17 = MMBF40/11(AG-F), 18 = MMBF39/10 (AG-G), 19 = MMBF35/10 (AG-P), 20 = CMM-1315 (AG-R), 21 = CMM-13 (*Fusarium oxysporum*), 22 = CMM-3065 (*Sclerotium rolfsii*), 23 = CMM-4047 (*Macrophomina phaseolina*) and 24 = water (ddH_2O). **Table S1:** jph70162-sup-0010-TableS1.docx. *Fusarium oxysporum*, *Macrophomina phaseolina*, and *Sclerotium rolfsii* isolates used in this study. **Table S2:** jph70162-sup-0011-TableS2.docx. *Rhizoctonia solani* (Rs) and binucleate *Rhizoctonia* (BNR) reference isolates used in the phylogenetic analysis.