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Transmission of the M2 double-stranded RNA in *Rhizoctonia solani* anastomosis group 3 (AG-3)

Nikki D. Charlton
Marc A. Cubeta[1]
Department of Plant Pathology, North Carolina State University, Raleigh, North Carolina, 27695-7616

Abstract: Horizontal transmission of the 3.57 kb M2 double-stranded RNA (dsRNA) between mycelia of somatically incompatible isolates of *Rhizoctonia solani* anastomosis group 3 (AG-3), an economically important pathogen of cultivated plants in the family Solanaceae, was investigated. Nine donor isolates of *R. solani* AG-3 containing the M2 dsRNA were paired on potato-dextrose agar with each of three different recipient isolates where the M2 dsRNA was absent. Reverse-transcription PCR (RT-PCR) was used to detect horizontal transmission of the M2 dsRNA via hyphal anastomosis from donor to recipient isolates by examining hyphal explants taken 3 cm from the hyphal interaction zone. PCR-RFLP genetic-based markers of two nuclear loci and one mitochondrial locus were used to confirm identity and transmission between donor and recipient isolates of *R. solani* AG-3. The frequency of transmission observed between 72 pairings of the eight donor and three recipient isolates was approximately 4% of the total pairings, and differences in the phenotype of the recipient isolates after acquisition of the M2 dsRNA via horizontal transmission were observed. To our knowledge this represents the first demonstration of transmission of dsRNA between genetically different individuals of *R. solani* confirmed with nuclear and mitochondrial markers. These results suggest that transmission can occur between somatically incompatible isolates of *R. solani* AG-3 but that maintenance of the dsRNA in the recipient isolates was not stable after repeated subculturing on nutrient medium.

Key words: Basidiomycete, horizontal transmission, mitochondrial markers, mycovirus, nuclear markers

INTRODUCTION

The occurrence of double-stranded RNA (dsRNA) viruses and virus-like elements in filamentous fungi and Oomycota is well documented and has been described in at least 150 species (Buck 1986, Deacon 2005). In the plant pathogenic soil fungus *Rhizoctonia solani* Kühn (teleomorph = *Thanatephorus cucumeris* Frank [Donk]) dsRNA (0.3–25 kb) have been found in isolates representing all anastomosis groups (AG) in the *R. solani* species complex (Bharathan and Tavantzis 1990, Lakshman and Tavantzis 1994, Robinson and Deacon 2002, Bharathan et al 2005). However the role that these dsRNA play in the biology and ecology of *R. solani* is not well understood.

dsRNA have been the subject of investigation since their initial discovery in *R. solani* anastomosis group 1 (AG-1) by Castanho and Butler (1978a, b) because of their effect on the parasitic activity of the fungus and potential for biological control of Rhizoctonia diseases of plants. Castanho and Butler (1978a, b) introduced the term ''Rhizoctonia decline'' to describe an isolate of *R. solani* AG-1 (189) with dsRNA that had a decreased growth rate and produced few to no sclerotia on nutrient medium. These researchers subsequently demonstrated vertical transmission of the dsRNA into single basidiospore progeny of isolate 189 and horizontal transmission via vegetative mycelium between somatically compatible isolates (e.g. clones) obtained by hyphal tip subculturing. In these experiments transmission of dsRNA from the donor to the recipient isolate produced a colony that exhibited the Rhizoctonia decline phenotype. Unlike the abnormal phenotypic characteristics of Rhizoctonia decline observed by Castanho and Butler other studies with *R. solani* found that the presence of dsRNA did not have any phenotypic effects on their growth or morphology on nutrient medium (Finkler et al 1985, Kousik et al 1994). Finkler et al (1985) were able to demonstrate horizontal transmission of dsRNA via hyphal fusion between isolates of *R. solani* AG-4 with benomyl as a selectable marker in experiments to evaluate heterokaryon formation. Tavantzis and colleagues also were able to transmit dsRNA between hyphal tip subcultures from isolate Rhs1AP of *R. solani* AG-3 to create isogenic strains to better understand how the presence of dsRNA influenced the ability of the fungus to cause disease on potato (Tavantzis 1994, Jian et al 1997, Lakshman et al 1998, Tavantzis et al 2002). In these experiments a 3.57 kb dsRNA called M2 was identified that was associated with the reduced ability of *R. solani* AG-3 to cause disease on potato (=hypovirulence). Although these studies have demonstrated transmission of dsRNA

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[1] Corresponding author. E-mail: marc_cubeta@ncsu.edu

TABLE I. Host origin, occurrence of the M2 double-stranded RNA (dsRNA) and donor/recipient status of isolates of *Rhizoctonia solani*

Isolate	Host	Origin[a]	M2 dsRNA[b]	Note
Tom7b	*Solanum lycopersicum*	NC	−	Recipient
T1[c]	*Nicotiana tabacum*	NC	+	Donor
Rs3	*Solanum tuberosum*	ME	+	Donor
Rs47	*Solanum tuberosum*	ME	−	Recipient
Rs60	*Solanum tuberosum*	WI	+	Donor
Rs84	*Solanum tuberosum*	WI	+	Donor
Rs87	*Solanum tuberosum*	ME	+	Donor
Rs109	*Solanum tuberosum*	NC	+	Donor
Rs137	*Solanum tuberosum*	NC	+	Donor
Rs189	*Solanum tuberosum*	NC	+	Donor
Rs206	*Solanum tuberosum*	NC	+	Donor
Rs220	*Solanum tuberosum*	NC	−	Recipient

[a] NC = North Carolina, ME = Maine, WI = Wisconsin.
[b] Detection of the M2 dsRNA based on reverse transcription PCR using primers P35 and P36 that result in amplification of approximately 1100 bases of the 3′ region.
[c] T1 is an isolate of *R. solani* AG-4.

between closely related isolates of *R. solani* (often clones of the same parental isolate) little is known about whether horizontal transmission of dsRNA via hyphal anastomosis can occur between clonal or closely related isolates of *R. solani*.

In this study we examined horizontal transmission of the M2 dsRNA via hyphal anastomosis in somatically incompatible isolates of *R. solani* AG-3. It has been shown that field populations of *R. solani* AG-3 on potato are genetically diverse and have a structure that includes both recombination and clonality (Ceresini et al 2002). Reverse-transcription PCR (RT-PCR) was used to detect horizontal transmission of the M2 dsRNA via hyphal anastomosis from donor to recipient isolates. PCR-RFLP nuclear and mitochondrial markers were used to confirm transmission and to distinguish between donor and recipient mycelia.

MATERIALS AND METHODS

Isolates.—All isolates of *Rhizoctonia solani* AG-3 and one isolate of *R. solani* AG-4 used in this study are listed (TABLE I). Isolates were stored by placing a 5 mm diam plug of the actively growing culture in 500 µL of potato-dextrose broth (PDB) in a 2.0 mL cryogenic vial (Corning®) for 24–48 h. After incubation 500 µL of 50% glycerol (v/v) was added to the vials, mixed with a vortex and stored at −80 C. Isolates were cultured on potato-dextrose agar (PDA) at 24 C before each experiment. Eight isolates of *R. solani* AG-3 from potato (*Solanum tuberosum* L.) (Rs3, Rs60, Rs84, Rs87, Rs109, Rs137, Rs189 and Rs206) and one *R. solani* AG-4 from tobacco (*Nicotiana tabacum* L.) (T1) that contained the M2 dsRNA were chosen as donor isolates of dsRNA. Two isolates of *R. solani* AG-3 from potato (Rs47 and Rs220) and one isolate from tomato (*Solanum lycopersicum* [L.] Karst. ex. Farwell) (Tom7b) were chosen as recipient isolates. M2 dsRNA was not detected in the recipient isolates based on reverse-transcription PCR (RT-PCR). Three replicates of each donor isolate were paired with each recipient isolate to study horizontal transmission of the M2 dsRNA between isolates of *R. solani* as described below.

Somatic compatibility.—Somatic compatibility was determined by pairing donor isolates with the recipient isolates on PDA amended with 1% charcoal (Butler and Bolkan 1973) following the procedure of MacNish et al (1997) to determine vegetative hyphal interactions. Self-pairings were used as controls. The paired isolates were incubated at 24 C for 4–5 d, and macroscopic interactions were observed. Somatically incompatible reactions were scored as weak, intermediate or strong based on the width of the reaction line that occurred in the hyphal interaction zone (FIG. 1). The somatic hyphal interactions also were examined microscopically with the following procedure. Isolates were grown on PDA for 3 d at 25 C in the dark. Mycelial plugs (5 mm diam) taken from the advancing margin of each isolate were paired 2 cm apart on glass slides covered with 2% water agar, placed in a plastic box (100 × 200 × 30 mm^3) on a sterile paper towel moistened with sterile deionized water and incubated at 25 C in the dark for 24–48 h. Three replicates were prepared from each pairing combination. After 24–48 h the overlapping portion of mycelium was stained with one drop each of 0.6% phenosafranin (No. 199648, Sigma-Aldrich, St Louis, Missouri) and 3% KOH. The somatic interactions were determined by observation at 400× magnification. Hyphal interactions were scored according to the scale developed by Carling (1996), where C0 indicates no hyphal fusion, C1 involves hyphal contact with cell wall but no cytoplasmic fusion, C2 indicates cell wall and cytoplasmic fusion accompanied by pore formation and death of adjacent cells and C3 indicates cell wall and cytoplasmic fusion with no death of fused cells. Self-pairings served as controls.

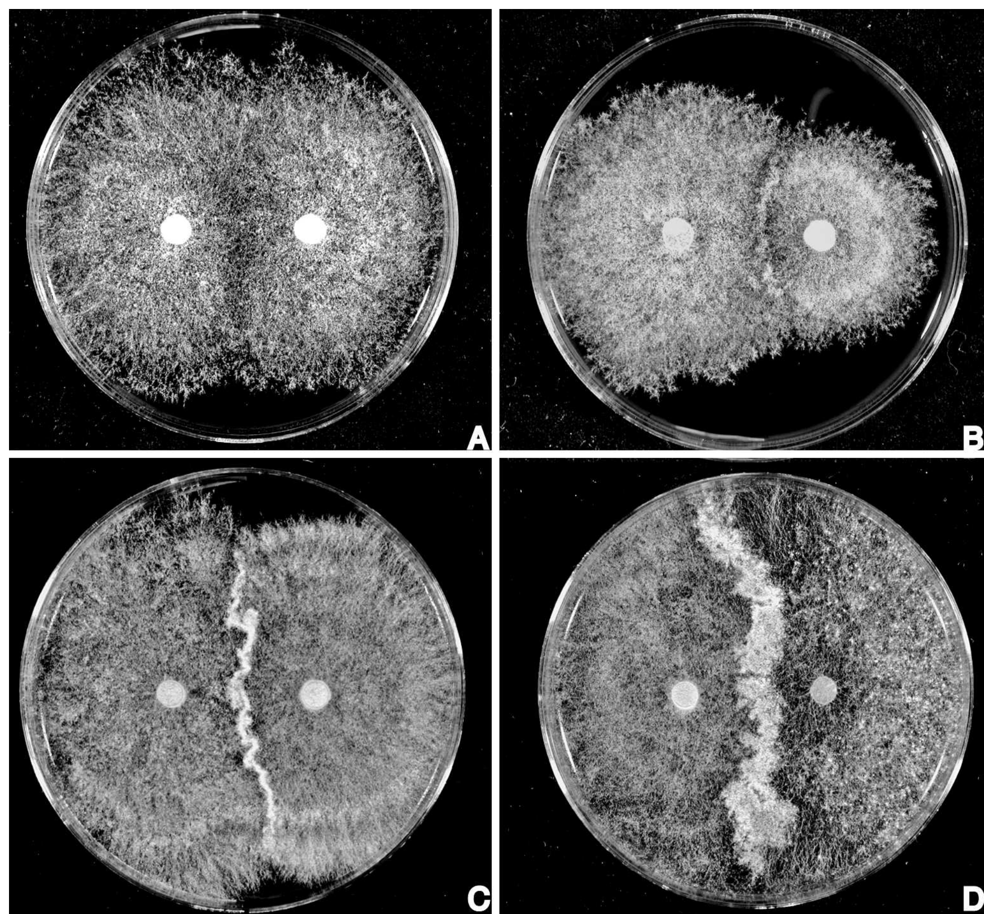

FIG. 1. Range of compatibility responses of *Rhizoctonia solani* anastomosis group 3 on potato-dextrose agar amended with 1% charcoal. a. Somatically compatible interaction. b. Somatically incompatible weak interaction. c. Somatically incompatible intermediate interaction. d. Somatically incompatible strong interaction.

Horizontal transmission.—To determine horizontal transmission of dsRNA via fusion of vegetative mycelium one donor and one recipient isolate were paired on PDA by placing 5 mm agar disks taken from actively growing cultures from each isolate 3 cm apart. Plates were incubated at 24 C for 4 wk in the dark. Three replicates of each pairing were made. A total of 81 pairings were conducted with eight donor and three recipient isolates of *R. solani* AG-3 and one donor isolate of *R. solani* AG-4. Because hyphae of isolates of *R. solani* AG-3 and AG-4 do not recognize and fuse with each other these pairing were included in our studies as negative controls. Self-pairings were used as controls for comparison purposes to make observations of any changes in phenotype. A 5 mm × 25 mm piece of agar (explant) taken 3 cm from the hyphal interaction zone was transferred to PDA. Plates were incubated 3–4 d at 24 C. DNA and RNA were extracted from isolates, assessed for the M2 dsRNA with RT-PCR and for nuclear and mitochondrial markers with PCR-RFLP methods to distinguish between donor and recipient isolates as described below.

Cultivation of fungal tissue for extraction of nucleic acids.—Isolates were grown in 25 mL of potato-dextrose broth (PDB). After incubation 3–4 d at 24 C mycelial mats were washed three times with sterile deionized water, lyophilized and stored at −80 C. Total genomic RNA was extracted from approximately 50–100 mg of ground mycelium with TRIZOL® Reagent (Invitrogen) according to manufacturer's instructions. Lithium chloride was used to improve RNA isolation by removing excessive amounts of carbohydrates (Salzman et al 1999, Liu C et al 2003). The RNA pellets were suspended in 40 μL of RNase-free water and stored at −20 C to be used in RT-PCR. Approximately 20 mg of ground mycelium was used in the DNA extraction method. DNA was isolated with QIAGEN® DNeasy Plant Mini Kit (QIAGEN, USA) according to manufacturer's instructions and extracted DNA was stored at −20 C.

Reverse-transcription polymerase chain reaction.—Total RNA was denatured at 100 C for 1 min in the presence of the gene-specific primer P36 (5′-GGGGCTTCTGGCGGAAAGAA-3′) (Liu C et al 2003). Integrated DNA Technologies Inc. (Coralville, Iowa) synthesized and provided all oligonucleotide primers used in this study. Primer P36 was used in reverse-transcription of the total RNA for the 3′ end (bases 2437–3570) of the M2 dsRNA (GenBank accession number U51331) for synthesis of complementary DNA

TABLE II. Oligonucleotide primer pairs used to amplify regions of nuclear DNA and mitochondrial large ribosomal DNA (rDNA) and restriction endonucleases used for PCR-RFLP analyses of donor and recipient isolates of *Rhizoctonia solani* anastomosis group 3 (AG-3)

Primer	Primer sequence	Product size (bp)	Annealing temperature (C)	Enzyme
Nuclear[a]				
pP42F	5′-GTTTTTGTAGTGCACGGGGGT-3′	1970	57	*Hin*d III
pP42R	5′-ATTCAACGTCTGTCGGTACGG-3′			
pP46F	5′-ATTAGCCCTGACTGGGTCTCG-3′	3050	57	*Hin*d III
pP46R	5′-ACGATATTGCCTAGTGCGACC-3′			
pP83F	5′-TTGTGAACCTTACAAGTACCCGA-3′	1370	55	*Hin*d III
pP83R	5′-AAGTATTATTCTCTGCGGTTCGC-3′			
Mitochondrial				
ML5[b]	5′-CTCGGCAAATTATCCTCATAAG-3′	359	53	
ML6[b]	5′-CAGTAGAAGCTGCATAGGGTC-3′			

[a] Ceresini et al (2002).
[b] White et al (1990).

(cDNA). After denaturation the reaction was chilled immediately in an ethanol-ice bath. RNA was reverse-transcribed in a total volume of 20 µL with 200 U Superscript II™ Reverse Transcriptase (Invitrogen), 500 µM of each dNTP (Promega, Madison, Wisconsin) and 20 U RNase OUT (Invitrogen) at 42 C for 90 min. The reaction was heat inactivated at 70 C for 10 min. cDNA were stored at −20 C and used as a template for amplification with PCR.

PCR was performed in a total volume of 50 µL containing 2 µL-aliquot of undiluted cDNA template, 1.25 U *Taq* polymerase (QIAGEN Inc., USA), 10× reaction buffer containing 15 mM $MgCl_2$ (QIAGEN Inc., USA), 200 µM of each dNTP (Promega, Madison, Wisconsin), and 1 µM target-specific primers P35 (5′-GTCATTACATGCAGCT-TACC-3′) (Liu C et al 2003) and P36. The cycling parameters were an initial denaturation step for 1 min at 94 C, 35 cycles of denaturation at 94 C for 1 min, annealing at 55 C for 45 s, extension at 72 C for 1 min, followed by a final synthesis step at 72 C for 7 min. Amplicons were analyzed by gel electrophoresis with a 1% agarose gel in 0.5× Tris-Boric-EDTA (TBE) buffer. DNA fragments were viewed after staining with ethidium bromide by UV transillumination.

Nuclear and mitochondrial markers.—A region of the mitochondrial large ribosomal DNA (rDNA) subunit was amplified with the polymerase chain reaction (PCR) with the ML5 and ML6 primers developed by White et al (1990) to identify the cytoplasmic background of explant isolates. Nuclear markers (pP42, pP46 and pP83) developed by Ceresini et al (2002) were used to identify the nuclear background of explant isolates. The pP42F region has sequence similarity to the nuclear gene *lsc2p* from *Saccharomyces cerevisiae* Meyen ex E.C. Hansen encoding for beta subunit of succinyl-CoA ligase or to a similar gene from *Neocallimastix frontalis* (R.A. Braune) Vavra & Joyon ex I.B. Heath. The pP89 region has sequence similarity to the nuclear gene *crm1* from *Schizosaccharomyces pombe* Linder encoding for chromosome region maintenance protein 1 or exportin 1. pP46 represents an anonymous region of nuclear DNA whose putative function cannot be identified based on sequence similarity. (The primer sequences and annealing temperatures used to amplify mitochondrial rDNA and nuclear loci to distinguish between donor and recipient isolates of *R. solani* AG-3 after transmission are presented in TABLE II.)

PCR was performed in a total volume of 50 µL containing 1.5 µL of undiluted DNA, 1.25 U *Taq* polymerase (QIAGEN Inc., USA), 10× reaction buffer containing 15 mM $MgCl_2$ (QIAGEN Inc., USA), 200 µM of each dNTP (Promega, Madison, Wisconsin) and 1 µM target-specific primers. The cycling parameters were an initial denaturation at 94 C for 1 min, 35 cycles of denaturation at 94 C for 1 min, annealing at 55 C for 45 s, extension at 72 C for 1 min, followed by a final synthesis step at 72 C for 7 min. Annealing temperatures were 53, 55 or 57 C, depending on the T_m of the primers (TABLE II). Amplicons were analyzed by gel electrophoresis with a 1% agarose gel in 0.5× TBE buffer. DNA fragments were viewed after staining with ethidium bromide by UV transillumination.

To identify the polymorphic sites to differentiate donor, recipient and explant isolates aliquots of DNA amplified with PCR with pP42, pP46 and pP83 primer sets were digested with restriction endonucleases according to Ceresini et al (2002). The PCR-RFLP primers and enzymes chosen for each isolate were based on polymorphisms between the donor and recipient isolates. The PCR-RFLP and PCR products were analyzed on a 1% agarose gel in 0.5× TBE buffer and viewed after staining with ethidium bromide by UV transillumination.

RESULTS

Somatic compatibility.—All paired donor and recipient isolates were somatically incompatible. The de-

TABLE III. Macroscopic somatic interactions of paired donor and recipient isolates of *Rhizoctonia solani* on potato-dextrose agar amended with 1% charcoal

Donor isolate	Recipient isolate		
	Rs47	Rs220	Tom7b
T1	–[Ta]	–[T]	–[T]
Rs3	–[1]	–[3]	–[2]
Rs60	–[1]	–[3]	–[2]
Rs84	–[3]	–[3]	–[3]
Rs87	–[2]	–[3]	–[2]
Rs109	–[1]	–[3]	–[2]
Rs137	–[2]	–[2]	–[2]
Rs189	–[1]	–[2]	–[2]
Rs206	–[2]	–[2]	–[2]

[a] – represents somatic incompatibility, where –[1] represents a weak interaction between paired isolates with small areas of raised hyphae approximately 1–2 mm wide; –[2] represents an intermediate interaction between paired isolates with a distinct demarcation zone of raised hyphae approximately 2–4 mm wide; –[3] represents a strong interaction between paired isolates with a distinct demarcation zone of raised hyphae ≥ 5 mm wide; –[T] represents a distinct demarcation between isolates with a raised tuft of hyphae.

gree of incompatibility ranged from weak to strong as manifested by differences in the width of matted hyphae in the interaction zone on PDA amended with 1% charcoal (FIG. 1, TABLE III). All self-pairings were compatible and exhibited no demarcation between paired isolates (FIG. 1). The pairing between the *R. solani* AG-4 and AG-3 isolates resulted in the formation of a distinct demarcation and a raised tuft of hyphae that differed from the pairings between AG-3 isolates (MacNish et al 1997). All donor and recipient isolates paired on 2% water agar-covered glass slides were somatically incompatible and microscope hyphal interactions were presented as cell wall and cytoplasmic fusion accompanied by death of interacting and adjacent cells (C2 reaction, data not shown). All self-pairings were somatically compatible and presented as cell wall and cytoplasmic fusion of hyphae with no death of fused cells (data not shown). The pairing between the *R. solani* AG-4 and AG-3 isolates showed a C0 reaction.

Horizontal transmission.—Horizontal transmission of the M2 dsRNA occurred between three donor isolates from potato (Rs3, Rs84 and Rs109) and one recipient isolate from tomato (Tom7b) (TABLE IV). The M2 dsRNA was detected in three explant isolates, Tom7b-Rs3C, Tom7b-Rs84A and Tom7b-Rs109C, based on RT-PCR amplification with P35 and P36 primers. These isolates were analyzed further with nuclear and mitochondrial markers to differentiate between the

TABLE IV. Transmission of the M2 double-stranded RNA (dsRNA) from donor to recipient isolates of *Rhizoctonia solani*

Donor isolate	Recipient isolate		
	Rs47	Rs220	Tom7b
T1	0/3[a]	0/3	0/3
Rs3	0/3	0/3	1/3
Rs60	0/3	0/3	0/3
Rs84	0/3	0/3	1/3
Rs87	0/3	0/3	0/3
Rs109	0/3	0/3	1/3
Rs137	0/3	0/3	0/3
Rs189	0/3	0/3	0/3
Rs206	0/3	0/3	0/3

[a] Frequency of dsRNA transmission is expressed as the number of explants where the M2 dsRNA was detected in three replicate pairings of donor and recipient isolate.

donor and recipient isolate origin. The PCR-RFLP nuclear markers pP42-*Hin*d III, pP46-*Hin*d III and pP83-*Hin*d III and the ML5-ML6 region of the large mitochondrial rDNA were used to confirm each successful transmission (FIG. 2). Molecular markers showed consistently that the nuclear and mitochondrial content of the explant isolates were of the recipient isolate, and in no case did we detect any evidence of mycelia through-growth based on nuclear or mitochondrial transmission of DNA from the donor mycelium.

The three explants where the M2 dsRNA was detected were subcultured a second time after approximately 2–3 wk and observed for phenotypic changes and reassessed for the M2 dsRNA. After the repeated subculturing, the cultures showed sectoring with phenotypic changes such as ''fluffy'' colony morphology and increased pigmentation. The M2 dsRNA was not detected after the repeated subculturing of the explant isolates.

DISCUSSION

Because of the intimate biotrophic association of a dsRNA virus with a fungal host their maintenance and spread in a field population is dependent largely on their ability to move within and between isolates. Because fungal viruses do not have an extracellular phase they require cell-to-cell contact and cytoplasmic mixing for virus transmission between cells or individuals (Buck 1986, Strauss and Strauss 1988). Horizontal transmission of dsRNA viruses has been shown to occur in several species of filamentous fungi including *Cryphonectria cubensis* (Bruner) C.S. Hodges (van Heerden et al 2001), *C. parasitica* (Murill) Barr (Friese et al 1992, Liu and Milgroom

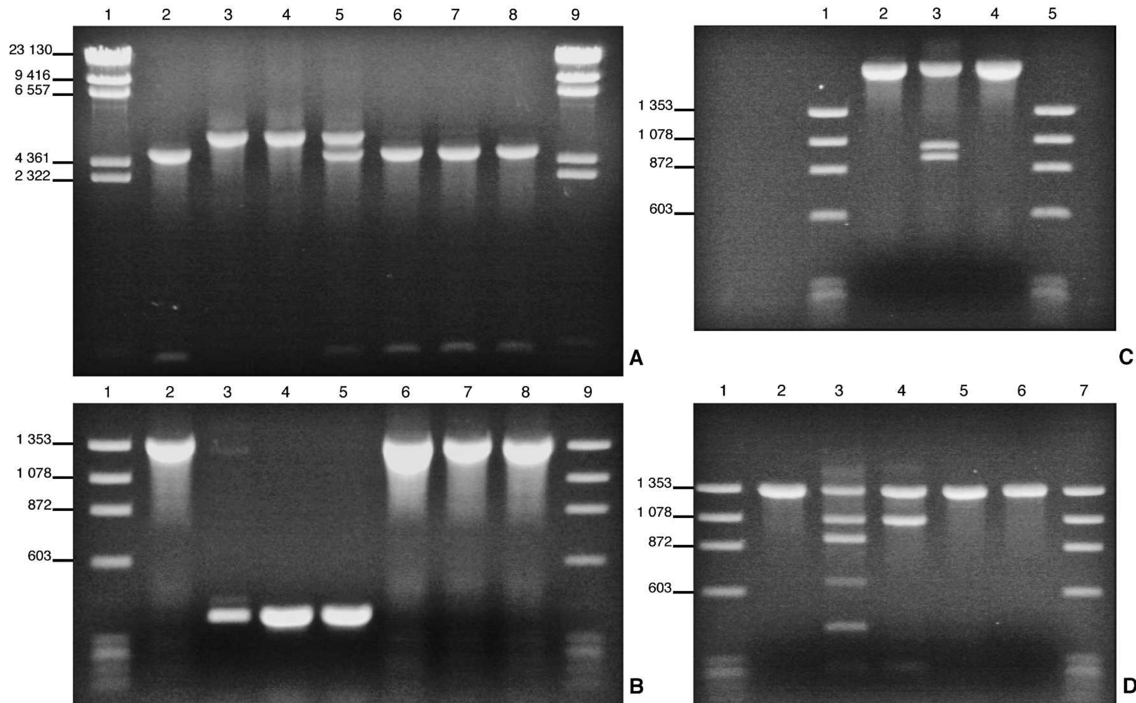

FIG. 2. Restriction fragment length polymorphism analysis of amplified DNA with locus specific primers (pP42-*Hin*d III, pP46-*Hin*d III, pP83-*Hin*d III) and polymorphism detection in the ML5-ML6 region of the mitochondrial large rDNA; sizes of bands (bp) are indicated on the left. a. RFLP analysis with the locus specific primer pP46 digested with *Hin*d III. Lanes 1 and 9 show λ DNA digested with *Hin*d III. Lanes 2-8 show RFLP fragments from isolates Tom7b, Rs3, Rs84, Rs109, Tom7b-Rs3C, Tom7b-Rs84A, and Tom7b-Rs109C. b. Polymorphism detection in the ML5-ML6 region of the mitochondrial large rDNA. Lanes 1 and 9 show ϕX174 DNA digested with *Hae* III. Lanes 2-8 show DNA fragments from isolates Tom7b, Rs3, Rs84, Rs109, Tom7b-Rs3C, Tom7b-Rs84A, and Tom7b-Rs109C. c. RFLP analysis of amplified DNA with the locus specific primer pP42 digested with *Hin*d III. Lanes 1 and 5 show ϕX174 DNA digested with *Hae* III. Lanes 2–4 show RFLP fragments from isolates Tom7b, Rs3, and Tom7b-Rs3C. d. RFLP analysis of amplified DNA with the locus specific primer pP83 digested with *Hin*d III. Lanes 1 and 7 show ϕX174 DNA digested with *Hae* III. Lanes 2–6 show RFLP fragments from isolates Tom7b, Rs84, Rs109, Tom7b-Rs84A, and Tom7b-Rs109C.

1996, Liu Y.C. et al 2003), *Heterobasidion annosum* (Fr. : Fr.) Bref. (Ihrmark et al 2002), *Sclerotinia minor* Jagger, *S. sclerotiorum* (Lib.) de Bary (Melzer et al 2002), and *Helicobasidium mompa* Tanaka (Suzaki et al 2003, Suzaki et al 2005).

The horizontal transmission of dsRNA viruses via vegetative mycelia is controlled by vegetative or somatic incompatibility systems (Milgroom 1999). As the number of allelic differences at the vegetative incompatibility (*vic*) genes increases the frequency of virus transmission via fusion of vegetative mycelium between isolates of *C. parasitica* decreases (Liu and Milgroom 1996, Cortesi et al 2001). These allelic differences, coupled with the rate at which programmed cell death of interacting hyphal cells occurs during an incompatible interaction, are thought to represent an evolved defense mechanism to limit virus transmission in filamentous fungi (Biella et al 2002). However these incompatibility systems do not always restrict virus transmission (Biella et al 2002, Cortesi et al 2001).

The frequency of horizontal transmission of dsRNA can vary within and among fungi species. However few studies have examined the frequency of horizontal transmission of dsRNA in the Basidiomycota, where less is known about the genetics of somatic incompatibility systems than in the Ascomycota. Ihrmark et al (2002) showed that horizontal transmission of dsRNA in somatically incompatible isolates of *H. annosum* occurred in 89 of 149 isolates paired (59.7%). The frequency of transmission was dependent on the specific combination of donor and recipient isolates paired that ranged 0–100%. Transmission of dsRNA between isolates of *H. mompa* belonging to different mycelial compatibility groups occurred in 27 of 360 donor and recipient pairings (7.5%) (Suzaki et al 2005). We were able to detect transmission of the M2 dsRNA between somatically incompatible isolates of *R. solani* AG-3. However the frequency of dsRNA transmission between mycelia of *R. solani* AG-3 was low and occurred in only three of 72 donor and recipient pairings (∼4% of the total

pairings) conducted under laboratory conditions. We also did not observe a relationship between the degree of somatic incompatibility determined on nutrient medium and transmission of the M2 dsRNA. However the limited number of successful transmissions might have prevented our ability to adequately interpret these relationships. Therefore further studies are needed to identify genes that govern somatic recognition in *R. solani* AG-3 and to determine their role in transmission of dsRNA.

Somatic recognition systems in *R. solani* play an important role in the ecology and transmission of dsRNA. We chose to investigate the transmission of the M2 dsRNA between somatically incompatible isolates because it represents the most well characterized dsRNA in *R. solani* and has been hypothesized to regulate the parasitic and saprotrophic activity of *R. solani* AG-3 (Tavantzis 1994, Jian et al 1997, Lakshman et al 1998, Tavantzis et al 2002, Charlton et al 2006). In addition studies by Ceresini et al (2002) provided multilocus genotype information on isolates of *R. solani* AG-3 for developing DNA-based nuclear markers to complement mtDNA markers needed to assess accurately the identity of donor and recipient isolates used in our transmission experiments.

To our knowledge this is the first report of horizontal dsRNA transmission between somatically incompatible isolates of *R. solani* representing different multilocus genotypes confirmed with RT-PCR and nuclear and mitochondrial markers. In three previous studies with *R. solani* horizontal transmission of dsRNA was demonstrated between closely related isolates of *R. solani* (often clones of the same parental isolate) (Castanho and Butler 1978a, Finkler et al 1985, Tavantzis et al 2002). Castanho and Butler (1978a) were unable to transmit the dsRNA and disease phenotype from isolate 189 exhibiting Rhizoctonia decline to four field isolates. The authors suggested that transmission was ''isolate specific'' and that their inability to transmit dsRNA might be related to somatic incompatibility mechanisms. In the studies of Finkler et al (1985) it was not clear whether horizontal transmission was demonstrated in genetically different and somatically incompatible isolates. In our studies mtDNA and nuclear markers were used to confirm that the M2 dsRNA was present in the recipient isolates. The mtDNA marker was critical for determining whether the M2 had moved from the cytoplasm of the donor isolate into the cytoplasm of the recipient isolate and that this movement was not a result of growth and introgression of the donor isolate onto the recipient isolate side of the plate. A similar approach was used to verify horizontal transmission of dsRNA in *H. annosum* (Ihrmark et al 2002).

The acquisition of the M2 dsRNA from three different isolates by isolate Tom7b produced explants that sectored on repeated subculturing and exhibited phenotypic changes such as a ''fluffy'' colony morphology with increased brown pigmentation compared to the original donor and recipient isolates. Except for the studies of Castanho and Butler (1978a) no changes in phenotype of *R. solani* following acquisition of a dsRNA have been reported. In our experience M2 dsRNA is extremely stable and has been maintained through both successive subculturing and hyphal tip isolation of the donor isolates used in this study (Charlton et al unpublished, data not shown). Tavantzis and colleagues have found similar results and have maintained cultures with the M2 and other dsRNA in host isolates of *R. solani* AG-3 for more than 12 y with no change in dsRNA content (Lakshman and Tavantzis 1994).

The M2 dsRNA was maintained in explant isolates at least 2–3 wk but was not detected thereafter. This data suggests that the M2 dsRNA might not be stable when transmitted into a different cytoplasm in the new fungal host environment. Perhaps factors or cellular mechanisms other than somatic incompatibility present in the fungal host might interfere with the ability of the transmitted dsRNA to replicate and persist within the new host. The concept of the viral quasispecies also provides a plausible explanation of our results. This concept posits that a population equilibrium or evolutionary stasis exists in a stable environment but equilibrium is disrupted when the environment changes (Domingo et al 1996). The survival of plant viruses is dependent on rapid adaptation to changing environments when being transmitted to a new host (Schneider and Roosinck 2001) and this concept might apply to fungal viruses as well. Schneider and Roosinck (2001) found that selection acted on the diversity of plant virus populations, which showed that these populations maintain levels of diversity that are specific to the host species. Future studies will need to include information regarding mixed fungal virus infections and how this influences transmission of dsRNA viruses and concomitant phenotypic effects on the fungal host.

The horizontal transmission of dsRNA between different genotypes might have different effects on the phenotype of filamentous fungi. For example phenotypic changes have been shown to occur in *C. parasitica* when transmission of dsRNA occurs, allowing visual observation of successful transmission and providing a powerful way to differentiate between isolates that either possess or lack dsRNA. Studies with *C. parasitica* have shown that there are differences in the hypovirulence-associated effects of related viruses among fungal host genotypes (Chen and Nuss 1999).

Transmission and phenotypic affects might be influenced by the viral and fungal host genomes. In this study horizontal transmission of the M2 dsRNA occurred among three donor isolates (Rs3, Rs84 and Rs109) and the same recipient isolate (Tom7b). Two of the three donor isolates (Rs84 and Rs109) possessed the same M2 dsRNA haplotype based on sequence analysis representing 3219 bp (Charlton et al unpublished data). The dsRNA haplotype may play an important role in transmission and replication in the fungal cell.

Because *R. solani* does not produce asexual spores another potential avenue for spread is via vertical transmission to basidiospores. Although Castanho and Butler (1978a) demonstrated vertical transmission via basidiospores in *R. solani* AG-1 we were unable to address vertical transmission in our experiments due to the difficulty of producing the teleomorph in culture.

Transmission might occur rarely under laboratory conditions but might occur in natural populations when mycelia interact. Carbone et al (2004) found that estimates of migration between vegetative incompatibility groups of *C. parasitica* were not reflective of the results from transmission experiments in the laboratory. Further studies are needed to clarify the ecological role of dsRNA in *R. solani* and to explain the frequency in natural populations. To better understand the role of the M2 dsRNA we need to be able to better transmit or eliminate the dsRNA among specific fungal genotypes of *R. solani* AG-3. The presence and absence of specific M2 dsRNA haplotypes in different fungal genotypes would allow for the characterization of phenotypic effects on the fungal host.

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