Front. Agric. China 2007, 1(4): 361–367
DOI 10.1007/s11703-007-0061-4

RESEARCH ARTICLE

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Identification of field isolates of *Rhizoctonia solani* to detect quantitative resistance in rice under greenhouse conditions

Abstract The rates of *in vitro* hyphal growth of *Rhizoctonia solani* isolates, and their pathogenicity were evaluated to identify *R. solani* isolates that are suitable to detect quantitative resistance in rice. The isolates of *R. solani* were purified from the infected rice and two grass species in Arkansas over three years. Among 200 *Rhizoctonia*-like isolates, 102 isolates were identified as *R. solani*, and confirmed using a ribosomal DNA internal transcribed spacers' marker. The rates of *in vitro* hyphal growth of the 102 *R. solani* isolates ranged from 1.17 to 1.89 mm/h, of which only 13.7% were significantly different from each other. The rates of *in vitro* hyphal growth of eight selected isolates were correlated with lesion lengths ($r = 0.86$ at $P = 0.005\ 9$ and $r = 0.93$ at $P = 0.000\ 1$) on the detached leaves of rice cultivars of Jasmine 85 (resistant) and M202 (susceptible), respectively. The eight isolates were selected based on the mean values of the maximal (1.89), median (1.54) and minimal (1.17) rates of hyphal growth. Two isolates that consistently exhibited significant differences in the rates of the hyphal growth were selected to examine the aggressiveness of isolates in micro-chambers. Using a micro-chamber, the slow growing isolates separated susceptible cultivars from moderately resistant cultivars better than the fast growing isolates. In contrast, the differences in disease reactions between both *R. solani* isolates were undetected using a standard field evaluation method. We suggest that the slow growing isolates are more useful than the fast growing isolates for detecting quantitative resistance with the micro-chamber method.

Keywords thanatephorus cucumeris, micro-chamber, hyphal growth rate, aggressiveness

Received June 26, 2007; accepted July 22, 2007

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1 Introduction

Rhizoctonia solani (teleomorph: *Thanatephorus cucumeris* (Frank) Donk) is a common soil-borne and broad host pathogen responsible for several economically important diseases on numerous species including rice (*Oryza sativa*) (Savary et al., 1995; Ogoshi, 1996; Shan et al., 2002; Ceresini et al., 2002a; Ceresini et al., 2002b). In rice, it causes sheath blight, which is one of the most important and widely distributed diseases worldwide (Sneh et al., 1991; Rush and Lee, 1992). Susceptible cultivars can experience up to 50% grain loss (Rush and Lee, 1992; Cu et al., 1996) in an environment conducive to severe sheath blight.

R. solani is known as a fast growing pathogen. The most effective strategy of the management for *R. solani* is based primarily on the application of fungicides that are likely hostile to our environment. Management of the disease through cultural methods has been generally challenging due to the lack of the host specificity of the pathogen, and the susceptibility of rotation crops. Although managing rice sheath blight disease through genetic resistance is the most economical, the quantitative nature of resistance (Pinson et al., 1995; Zou et al., 2000; Yasufumi et al., 2002), uneven distribution of the pathogens in the field, and lack of comprehensive knowledge in the pathogen's biology have often made resistance screening difficult under field conditions. The objectives of this study were to survey the field sheath blight pathogen, examine the rate of *in vitro* hyphal growth of *R. solani* isolates, and determine *R. solani* isolates that are useful in detecting minor differences in sheath blight resistance under greenhouse conditions.

2 Materials and methods

2.1 Sample collection and isolate purification

Rice plants showing symptoms of sheath blight were collected from fields in 19 Arkansas counties (Fig. 1).

Two samples from other grass species, *Echinochola crusgalli* (L) Beauv. and *Panicum dichotomiflorum* Michx were also included (Table 1). The 41, 18, and 23 samples were collected in 2001, 2002, and 2003, respectively. An isolate recovered from a rice sample was designated as *Rhizoctonia* from *Rice* (RR) followed by the year of collection and sample number. Isolates from the two grass species were designated as *Rhizoctonia* from *Grass* (RG) (Table 1).

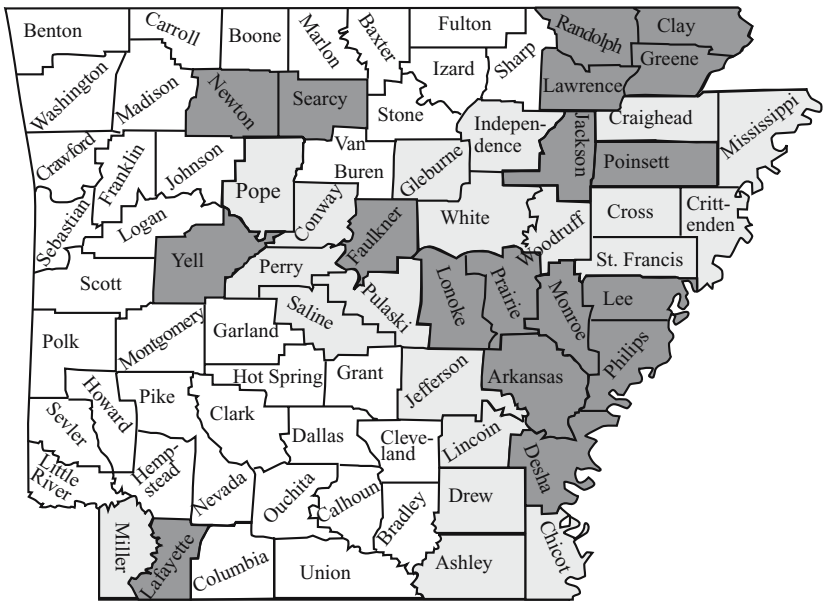
To purify *Rhizoctonia* species, approximately 5-cm-long pieces of the infected leaf or sheath were washed with running tap water on a strainer. The samples were disinfected by immersion in 3% w/v sodium hypochlorite for 1–2 min, and rinsed using sterile distilled water. The samples were removed aseptically, blotted dry, placed on Petri dishes containing water agar with tetracycline (0.005% w/v) (PDA_{Ttc}) and kept at 30°C in the dark. The cultures were examined under a compound microscope for an angle of hyphal branching. Those cultures with 45 or 90 degree angle hyphal branching were excised from the edge of the mycelia, and transferred to a new Petri dish containing PDA_{Ttc}. Each isolate was sub-cultured on PDA_{Ttc}, and verified by sclerotia production.

2.2 Identification of *R. solani* using a DNA marker

A DNA marker designed from a ribosomal DNA internal transcribed spacer was used to distinguish *R. solani* from other species from the *Rhizoctonia* genus. Genomic DNA was extracted using Wizard Genomic DNA purification kit from Promega (Promega Corp., Madison, WI). The ITS1-5.8S-ITS2 regions were amplified according to Johanson et al. (1998) and Fenille et al. (2003) with primer pairs GMRS-4 (5′-CGGTTCATCTGCATTTACCTT-3′) and ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′). After confirmation using the DNA-ITS marker, sclerotia from *R. solani* isolates were stored in sterile vials at room temperature (21°C–24°C) for short-term storage. Agar disks with mycelia were kept at –80°C in 20% glycerol and mycelia on sterile filter paper at –20°C in vials, respectively.

2.3 Tests on hyphal fusion (anastomosis)

Twelve *R. solani* isolates from rice collected in 2001 were selected randomly and tested for the anastomosis groups at the Department of Plant Pathology, University of Arkansas, Fayetteville. The two isolates from other grass species also were included (Table 1). A standard set of testers representing 13 anastomosis groups (ID Al 1-4, AG-B1), (ID521, AG-9), (ID CI, AG-8), (ID1529, AG-7), (NTA3-1, AG-6), (ID ST6-1, AG-5), ((ID AH-1, AG-4), (ID W14L, AG-3), (ID RI-64, AG2-2), (ID F56L, AG2-1), (ID M43, AG1-1C), (ID Cs-Ka, AG1-IA) and (ID SFBV-1, AG1-IB) was used. Isolates were grown in Petri dishes containing PDA_{Ttc} at 30°C in the dark. To obtain relatively thin cultures with consequent shallow depth of microscopic fields (23), mycelia agar plugs (~ 1 cm^2) from three-day-old cultures were excised and placed on a 2.5 cm × 8 cm sterile dialysis membrane (cat: 25225-251, VWR Scientific) on a 1.4% water agar plate. A tester isolate was placed at the center of a dialysis membrane, with two Arkansas isolates on either side. After 16–24 h incubation,



Note: Counties where diseased rice and grass samples were collected are shown in the dark. One sample was obtained from an unidentified county.

Fig. 1 The map of the state of Arkansas showing rice growing counties

Table 1 A summary of *R. solani* isolates from diseased rice and grass samples

Sample	Number of isolates	Source /cultivar/farm	County
RR0101[a)]	1	Unknown	Jackson
RR0102[a)]	1	Unknown	Arkansas
RG0102[a)]	1	Barnyard grass[b)]	Lonoke
RG0103[a)]	1	Fall panicum[c)]	Lawrence
RR0103[a)]	1	LGN 14A	Arkansas
RR0104[a)]	1	95 Kaybonnet	Arkansas
RR0105	1	Wells	Clay
RR0107[a)]	1	Cocodrie	Lawrence
RR0108	1	Unknown	Lawrence
RR0113[a)]	1	CL 18	Faulkner
RR0120[a)]	1	CFX 18	Faulkner
RR0125[a)]	1	Wells	Arkansas
RR0128[a)]	1	Cypress	Lafayette
RR0129[a)]	1	Cypress	Lawrence
RR0133	1	RS409	Arkansas
RR0134	1	Wells	Faulkner
RR0135[a)]	1	Unknown	Arkansas
RR0136	1	Drew	Newton
RR0137	1	Unknown	Lonoke
RR0138	1	Unknown	Monroe
RR0139	1	Unknown	Lonoke
RR0140	1	Unknown	Prairie
RR0141	1	94SB01	Prairie
RR0204	8	Unknown	Desha
RR0214	2	Unknown	Prairie
RR0215	4	Rull 24	Monroe
RR0217	2	Unknown	Unidentified
RR0303	1	Cocodrie	Phillips
RR0304	2	Bengal	Randolph
RR0305	3	Francis	Yell
RR0314	5	Wells	Searcy
RR0315	1	CL161	Greene
RR0316	2	Cocodrie	Lee
RR0318	2	Unknown	Lonoke
RR0319	14	CL161	Lonoke
RR0321	5	Wells	Yell
RR0322	21	Francis	Poinsett
RR0323	7	Cocodrie	Mississippi

Note: An isolate recovered from a rice sample was designated as *Rhizoctonia* from *Rice* (RR) followed by the year of collection, as 01 if collected in 2001. Isolates from grass species other than rice were designated as RG.
[a)] represents isolates tested for AG; [b)] for *Echinochola crusgalli* (L) Beauv; and [c)] for *Panicum dichotomiflorum* Michx.

the hyphal fusion was visualized under a bright field microscope (Nikon Eclipse E600). The hyphal attraction or fusion indicated the isolate AG (2).

2.4 Rate of *in vitro* hyphal growth

A total of 102 *R. solani* isolates having PCR product characteristics of *R. solani* (Johanson et al., 1998) were selected to examine the rate of *in vitro* hyphal growth. To determine the rate of hyphal growth, an agar disk with mycelia (7-mm diam.) from a three-day-old culture grown on PDA_{Ttc} was excised with a 1-mL Eppendorf pipette tip. The agar disk with mycelia was placed in the center of a Petri dish and was incubated in a culture-room with 12/12 h florescence (light/night) at 24°C. The Petri dishes were completely randomized in three replications. The experiments were repeated twice. Data were recorded on diameter (D_1) of the initial agar disk with mycelia, the initial time (T_1) at which the agar disk with mycelia was introduced, diameter (D_2) of the hyphal growth after (24 ± 3) h, and the time (T_2) at which D_2 was measured. Rate (R) was calculated as difference in the diameter of hyphal growth over the time ($R = (D_2 - D_1)/(T_2 - T_1)$. Resulting data were analyzed using version 8.2 SAS (SAS Institute, Cary, NC) and means were compared using Tukey-Kramer test at $P = 0.05$. Eight isolates were selected based on the maximal (1.89), median (1.54), and minimal (1.17) hyphal growth rate mean values, respectively (Table 2). The selected isolates were three fast growing (RR0321-4-, RR0319-8, RR0105-1), two intermediate (RR0305-27, RR0316-1), and three slower growing (RR0316-1, RR0140-1, RR0141-1) (Table 3).

Table 2 Rate of *in vitro* hyphal growth (mm/h) of 16 out of 102 isolates of *R. solani*

Isolate[a)]	Expt 1	Expt 2	Expt 3	Mean /mm · h^{-1} [b)]
RR0321-4	1.95	1.90	1.83	1.89 a
RR0319-8	1.95	1.95	1.64	1.85 ab
RR0105-1	1.83	1.75	1.83	1.80 abcd
RR0323-8	1.69	1.87	1.79	1.78 abcd
RR0214-4	1.71	1.93	1.68	1.77 abcd
RR0319-4	1.57	1.84	1.90	1.77 abcd
RR0314-1	1.84	1.76	1.70	1.77 abcd
RR0318-4	1.81	1.85	1.65	1.77 abcd
RR0319-3	1.69	1.74	1.88	1.77 abcd
RR0305-27[c)]	1.43	1.71	1.50	1.54 abcdef
RR0103-1[c)]	1.39	1.68	1.55	1.54 abcdef
RR0139-1	1.08	1.50	1.13	1.23 cdef
RR0101-1	1.44	1.09	1.09	1.21 def
RR0316-1	1.14	1.18	1.29	1.20 def
RR0140-1	1.19	1.27	1.11	1.19 ef
RR0141-1	1.19	1.22	1.12	1.17 f

Note: [a)] means that an isolate recovered from a rice sample was designated as *Rhizoctonia* from *Rice* (RR) followed by the year of collection sample and entry numbers; [b)] indicates that means followed by the same letter are not significantly different at 5% level, and means represent three runs each in three replications; [c)] means that the remaining 86 isolates were not significantly different from one another and were similar to RR0305-27 and RR0103-1. More than one sub-sample was obtained from highly infected samples.

2.5 Evaluation of aggressiveness

2.5.1 Detached leaf method

The detached leaf method was first developed to evaluate the aggressiveness of the isolates in the lab (Venu et al., 2007). Rice plants were grown to the late tillering stage (V11) (Counce et al., 2000) and leaves were cut into 16-cm-long segments. The leaf segments were placed in 243 mm × 243 mm × 18 mm Petri dishes (Cat: 29431000, BioAssay Dish, PGC Scientific) containing sterilized and uniformly pre-wetted filter paper (225 cm × 225 cm) (3 MM Whatman). Agar disks with mycelia (7 mm diam.) from three-day-old mycelia grown on PDA_{Ttc} were excised with a 1-mL Eppendorf tip and placed face down at the middle

Table 3 Means of lesion lengths (mm) produced by eight isolates of *R. solani* on detached leaves of cultivars Jasmine (resistant) and M202 (susceptible)

Isolate's *in vitro* hyphal growth[a)]	Lesion length /mm							
	M202				Jasmine 85			
	Expt. 1	Expt. 2	Expt. 3	Mean[b)]	Expt. 1	Expt. 2	Expt. 3	Mean[b)]
Fast-1	109.93	127.16	126.85	121.31 ab	86.86	69.33	56.91	71.00 def
Fast-2	104.58	104.38	120.62	109.86 ab	57.29	64.74	66.16	62.70 ef
Fast-3	96.89	112.49	113.93	107.77 abc	56.14	50.05	52.47	52.90 efg
Intermediate-1	96.72	108.27	95.86	100.28 abc	55.49	43.87	59.42	52.90 efg
Intermediate-2	73.85	99.19	102.62	91.89 bcd	42.99	43.80	42.99	43.30 fgh
Slow-1	67.89	96.79	73.00	79.23 cde	42.27	51.28	41.54	45.00 fgh
Slow-2	61.27	76.57	72.91	70.25 def	33.35	29.46	29.54	30.80 gh
Slow-3	58.85	58.47	38.23	51.85 efg	27.21	7.87	16.99	17.40 h

Note: [a)] means Fast-1 = RR0321-4, Fast-2 = RR0319-8, and Fast-3 = RR0105-1; Intermediate-1 = RR0305-27, and Intermediate-2 = RR0103-1; Slow-1 = RR0316-1, Slow-2 = RR0140-1, and Slow-3 = RR0141-1. [b)] indicates that means followed by the same letter in a column and between columns are not significantly different at 5% level. Means represent three experiments that were conducted in three replications. *In vitro* hyphal growth rate and *in vivo* mean lesion length on Jasmine 85 and M202 were positively correlated ($r = 0.86$ at $P = 0.005\ 9$ and $r = 0.93$ at $P = 0.000\ 1$, respectively).

of the abaxial surface of the leaf segment using sterile toothpicks. Treatments were placed on a laboratory bench under continuous cool white fluorescent light (10–20 $\mu Em^{-2} \cdot s^{-1}$) at 21°C–24°C. Control leaf segments were inoculated with sterile PDA_{Ttc} agar disks. The eight isolates were tested in three replications in three independent trials. The longest lesion length was measured (72 ± 3) h after inoculation using an electronic digital caliper (VWR 12777-830). The relationships between the rates of *in vitro* hyphal growth and lesion length were analyzed using SAS system for mixed models with version 8.2 SAS (SAS Institute, Cary, NC). Subsequently, a fast growing isolate (RR0321-4) and a slow growing isolate (RR0140-1) were selected for micro-chamber and field tests.

2.5.2 Micro-chamber method

A micro-chamber method described by Shrank et al. (2004) and Jia et al. (2007) was subsequently used to examine the disease reaction of RR0321-4 and RR0140-1. Seven rice cultivars including Jasmine 85 as a resistant control (Pinson et al., 1995) and Lemont (Rush et al., 1998) as a susceptible control were tested (Table 4). The micro-chamber tests on the seven cultivars using RR0321-4 and RR0140-1 were repeated once each with four replications. The data were analyzed using Statgraphics at $P = 0.05$. The two isolates were verified using 11 cultivars that included Jasmine 85 as a moderately resistant control and M202 and Lemont as susceptible controls (Table 6) at growth stage V4 to V5 (18-day-old seedlings) using the micro-chamber method. The experiment was run three times in three replications with all lesions and culm heights measured each time. Percentage of lesion lengths relative to culm heights was calculated. Data on the mean percentage of lesion length were transformed to arcsine square root values and analyzed using SAS system for mixed models with version 8.2 SAS (SAS Institute, Cary, NC). Cultivars showing a disease level significantly different from the susceptible cultivars were considered moderately resistant. Disease reactions to the sheath blight disease from these tests were compared to the field resistance by Yan et al. (2002).

2.5.3 Field evaluation

RR0321-4 and RR0140-1 were used to evaluate disease reactions using a standard field evaluation method. Rice

Table 4 Lesion length[a)] produced by a slow growing (RR0140-1) and a fast growing (RR0321-4) isolates on seven rice cultivars tested in a micro-chamber

Cultivar	RR0140-1 (slow growing)				RR0321-4 (fast growing)			
	Mean[b)] Expt. 1	Mean[b)] Expt 2	Grand mean	Grand mean rank[c)]	Mean[b)] Expt. 1	Mean[b)] Expt 2	Grand mean	Grand mean rank[c)]
Cypress	4.8 cd	6.5 c	5.7	5	7.1 bc	7.2 bc	7.2	5
Rosemont	8.4 e	5.7 cd	7.1	6	7.8 cd	8.7 de	8.3	6
Pecos	3.0 ab	3.8 a	3.4	2	5.9 ab	6.4 b	6.2	2
Cocodrie	3.8 bc	6.3 cd	5.1	3	7.2 c	8.1 cde	7.7	4
TeQing	5.5 d	5.6 b	5.6	4	7.1 bc	7.6 bcd	7.4	3
Lemont	9.0 e	8.3 d	8.7	7	8.3 d	8.9 e	8.6	7
Jasmine 85	2.3 a	3.4 ab	2.9	1	5.9 a	5.2 a	5.6	1

Note: [a)] means the scale of 0 to 9 is used where 0 is immune and 9 is the most susceptible. [b)] indicates that means within a column followed by the same letter are not significantly different at 0.05% level. Jasmine 85 = resistant control. Lemont = susceptible control. [c)] means that the lower the number in its rank, the more resistant the cultivar is to sheath blight disease.

cultivars were grown in ten replications in 2005 each in 3-row and 1-m long plots. Pellets carrying *Rhizoctonia solani* mycelia were prepared from rice flour in V-8 and corn flour in Kelgin following the procedure developed by F. Lee (personal communication). Pellets were dried enough at low heat to ensure floating. Pellets, 25–30 mL per plot were suspended between rows in the flooded rice at the booting stage. Inoculation was repeated once in four weeks. Disease scores were taken using a 0–9 scale from the middle row and data were analyzed using Statgraphics at $P = 0.05$.

3 Results

3.1 Samples and isolate identification

Eighty-two diseased rice samples were collected over three years (2001–2003), representing samples from nearly 50% of the rice producing counties in Arkansas (Fig. 1). From 39 samples of the 82 diseased samples, 200 *Rhizoctonia*-like fungi were recovered (Table 1), among which 102 isolates that amplified rDNA-ITS regions with about 650 bp were distinguished as *R. solani*. The remaining isolates were other sclerotia forming fungi likely *R. oryzae* or *R. oryzae-sativae* (data not shown). Twelve isolates from rice (RR0101-1, RR0102-1, RR0103-1, RR0104-1, RR0107-1, RR0113-1, RR0120-1, RR0125-1, RR0128-1, RR0129-1, RR0134-1, RR0135-1) and isolates from the two grass species (RG0102-1, RG0103-1) that were selected for AG analysis all belonged to AG1-IA (Table 1).

3.2 Rates of *in vitro* hyphal growth

The rates of *in vitro* hyphal growth among *R. solani* isolates ranged from 1.17 to 1.89 mm/h with no significant differences between 86.3% of the isolates (Table 2). Only five isolates (RR0139-1, RR0101-1, RR0316-1, RR0140-1, RR0141-1) were significantly slower than the two fast growing isolates (RR0321-4, RR0319-8), and nine (RR0321-4, RR0319-8, RR0105-1, RR0323-8, RR0214-4, RR0319-4, RR0314-1, RR0318-4, RR0319-3) were significantly faster than the two slow growing isolates (RR0140-1, RR0141-1). Rates of *in vitro* hyphal growth did not show any correlation with year of collection, sample type, sample origin, or habitat/county but the two fastest and the two slowest growing isolates were from samples collected in 2003 and 2001, respectively.

3.3 Association of rates of *in vitro* hyphal growth and aggressiveness

3.3.1 Detached leaf method

The eight selected *R. solani* isolates (RR0321-4, RR0319-8, RR0105-1, RR0305-27, RR0103-1, RR0316-1, RR0140-1, RR0141-1) all produced lesions on penultimate detached leaves of Jasmine 85 and M202 (Table 3). All the eight isolates showed significant differences in lesion length between Jasmine 85 and M202. Lesion lengths on Jasmine 85 and M202 were positively correlated ($r = 0.86$ at $P = 0.005\ 9$ and $r = 0.93$ at $P = 0.000\ 1$, respectively) to the rates of *in vitro* hyphal growth, indicating that faster growing isolates were more aggressive than the slow growing isolates.

3.3.2 Micro-chamber method

Lesions produced on one set of seven cultivars using RR0321-4 were longer than those produced by RR0140-1. The slow growing isolate separated the positive control from the negative control better than the fast growing isolate. Both isolates agreed in more than 70% of the cultivars' rank order of resistance/susceptibility to sheath blight disease in these tests (Table 4). In field tests on these cultivars, the slow growing isolate was as pathogenic as the fast growing isolate and grouped the cultivars into two categories as either more or less resistant than Jasmine 85. The rank of field resistance/susceptibility orders was similar to both isolates (Table 5). However, the disease readings on most cultivars using the fast growing isolate were higher than the slow growing isolate. The fast growing isolate (RR0121-4) tested on 18-day-old seedlings of 11 rice cultivars including Jasmine 85, Lemont and M202 produced longer lesions on all cultivars than the slow growing (RR0140-1) isolate. RR0121-4 was more aggressive making 71.4% of these cultivars known to be tolerant to sheath blight in the field by Yan et al. (2002) as susceptible as M202 or Lemont (Table 6). On the other hand, the responses of 90.9% of these eleven cultivars tested in the growth chambers were in agreement with the field disease levels using RR0140-1 suggesting that small differences in resistance to rice sheath blight could be easily detected using

Table 5 Mean of lesion length[a)] produced by a slow growing (RR0140-1) and a fast growing (RR0321-4) isolates on seven rice cultivars tested in the field

Rice cultivar	RR0140-1 (slow growing)		RR0321-4 (fast growing)	
	Mean of lesion length[b)]	Rank[c)]	Mean of lesion length[b)]	Rank[c)]
Cypress	2.5a	3	4.8b	5
Rosemont	5.4b	6	3.2a	4
Pecos	2.4a	2	2.7a	1
Cocodrie	5.2b	5	5.2b	6
TeQing	2.2a	1	2.7a	2
Lemont	7.5c	7	8.0c	7
Jasmine 85	2.8a	4	3.1a	3

Note: [a)] means the scale of 0 to 9 is used where 0 is immune and 9 is the most susceptible. [b)] indicates that means within a column followed by the same letter are not significantly different at a 5% level. Jasmine 85 = resistant control. Lemont = susceptible control. [c)] field experiment was conducted once in ten replications and disease score was taken from the middle row of three-row plot.

Table 6 Means of lesion length percentage produced by two *R. solani* isolates on seedlings of 11 rice cultivars in the micro-chamber as compared to field disease ratings

Cultivar	RR0140-1 /(Slow, 1.17 mm·h^{-1})				RR0321-4 /(Fast, 1.89 mm·h^{-1})				Field disease rate[a)]	
	Expt. 1	Expt. 2	Expt. 3	Mean[b)]	Expt. 1	Expt. 2	Expt. 3	Mean[b)]	Plant1	Plant 2
Jasmine 85[c)]	28.56	29.35	27.28	28.39 g	46.26	35.59	50.25	44.03 efg	2	3
CDR210[d)]	40.36	24.24	23.22	29.27 g	71.95	31.41	43.81	49.06 defg	4	5
4484[d)]	30.73	43.45	40.83	38.34 fg	90.70	72.61	64.16	75.82 bcde	3	5.8
4583[d)]	62.75	34.17	39.19	45.37 efg	75.27	78.12	76.46	76.62 bcde	3	5.5
4582[d)]	54.71	63.37	59.05	59.04 cdefg	90.13	76.81	78.73	81.89 abcd	3.5	5.3
GP-2[d)]	80.32	36.85	40.31	52.49 efg	95.32	100	100	98.44 a	3.3	5.8
Guinean Dao[d)]	72.50	75.33	90.39	79.41 bcde	95.02	87.73	97.77	93.50 ab	3	5.5
Lemont	64.91	65.81	85.95	72.22 bcdef	100	100	87.84	95.95 ab	7.8	7.7
Katy	83.33	90.11	96.71	90.05 ab	100	86.9	100	95.63 ab	6	6
Labelle	94.19	82.46	83.84	86.83 abc	100	96.23	100	98.74 a	7	7
M202[e)]	88.76	87.98	81.19	85.98 abc	100	89.78	87.19	92.32 ab	7	7

Note: [a)] means that the field disease rate on individual plants was based on 0–9 scale (0 is immune and 9 is the most susceptible), and obtained from Yan et al. (2002). [b)] indicates that means followed by the same letter are not significantly different at 0.05% level based on arcsine square root transformed values using Tukey's test. Means represent three runs each with three replications. Lesion lengths are measured in millimeters. [c)] represents Jasmine 85 = resistant control. [d)] represents Chinese rice germplasm. [e)] represents M202 = susceptible control.

the slow growing isolates in micro-chambers at the seedling stage.

4 Discussion

Genetic resistance is the most economical method of managing rice diseases including sheath blight disease. However, the quantitative nature of resistance (Pinson et al., 1995; Zou et al., 2000; Yasufumi et al., 2002), and the uneven distribution of the pathogen in the field have made resistance screening for rice sheath blight more difficult. In this study we purified 102 *R. solani* isolates from the fields of Arkansas, the major state of rice production in the USA to examine disease reactions under greenhouse conditions. Their growths *in vitro* were determined, and the results allowed us to select the fungal isolates for estimating pathogenicity under greenhouse conditions. It was shown that the aggressiveness of each isolate could be predicted based on the speed of growth in Petri dishes. The fast growing *R. solani* isolates were more aggressive than the slow growing isolates. Both the slow and the fast growing isolates separated disease reactions from resistant Jasmine 85 and very susceptible M202 using both detached leaves (Venu et al., 2007) (Table 3) and micro-chamber methods (Jia et al., 2007). However, the fast growing isolate failed to distinguish minor differences of disease reactions of other cultivars. On the other hand, the slow growing isolates were found to be extremely useful for disease evaluation in the greenhouse. In the micro-chamber study, the slow growing isolate (RR0140-1) produced disease reactions that conformed with field disease reactions in ten of the 11 tested rice cultivars. When testing the seven rice cultivars in a micro-chamber using the slow growing isolate, the disease severity on all cultivars was less than on those tested using the fast growing isolate. Moreover, the positive and the negative controls were well separated using the slow growing isolate than the fast growing isolate. The fast growing isolate was more aggressive and showed more disease lesions than their actual reactions in the field. Results from both detached leaves and the micro-chamber studies suggest that the slow growing isolate is useful in detecting small quantitative resistance to sheath blight in rice cultivars under greenhouse conditions.

R. solani is a necrotrophic pathogen attacking many green plants on earth (Akino and Ogoshi, 1995; Savary et al., 1995; Ogoshi, 1996; Shan et al., 2002; Ceresini et al., 2002a; Ceresini et al., 2002b). The control of diseases has relied on the uses of pesticide and partial resistance genes in integrated cultural management. A number of quantitative resistance genes have been identified but the complete resistance to this pathogen has not been identified from cultivated rice (Pinson et al., 1995; Zou et al., 2000; Yasufumi et al., 2002). Jasmine 85 (Marchetti et al., 1998) is the only cultivar among all the cultivars tested, showing the strongest resistance to *R. solani* using evaluation techniques of both detached leaves and micro-chambers in the greenhouse and field. The challenges still lie ahead in identifying more resistance genes from rice germplasm worldwide. Field evaluation has been effective in evaluating disease reactions; however, field evaluation takes 4–7 months, and is limited to rice production areas. Often, it is an inefficient hit-and-miss process. Thus, the accurate and rapid evaluation of disease reactions to *R. solani* is the bottleneck of rice breeding programs. Greenhouse tests using appropriate isolates identified from this study should speed up the germplasm screening efforts worldwide.

Acknowledgements We thank Dr. Tom Popham and Mr. Howard Black for statistical analysis, Ms. Angela Rosencrantz, for preparation of the manuscript and Dr. Wengui Yan for providing rice seeds, and Melissa H. Jia for proofreading the manuscript. Technical support from the staff members of Molecular Plant Pathology Laboratory of Dale Bumpers National Rice Research Center and financial support from the University of Arkansas Rice Research and Promotion Board also are appreciated.

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