Anastomosis Groups and Pathogenicity of *Rhizoctonia solani* Isolates from Brazil

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ABSTRACT

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Anastomosis groups (AG) of 130 *Rhizoctonia solani* isolates obtained from 31 plant species found in 11 states of Brazil were determined. Fifty-nine isolates were assigned to AG-4, 10 to AG-3, 42 to AG-2, and 11 to AG-1. Eight isolates did not anastomose with any of the tester strains or among themselves, and no isolate was assigned to AG-5. Pathogenicity of 35 isolates from different anastomosis groups on hypocotyls and/or leaves of kidney bean, soybean, red pepper, radish, sugar beet, and cabbage varied considerably, from nonvirulent to highly virulent. In general, anastomosis groups lacked host specificity. The optimum growth temperature for isolates tested varied from 20 to 30 C.

Rhizoctonia solani Kühn, anamorph of *Thanatephorus cucumeris* (Frank) Donk, is a common pathogen of various commercially grown crops in Brazil. The fungus is troublesome on cabbage (*Brassica oleracea* var. *capitata* L.), lettuce (*Lactuca sativa* L.), potatoes (*Solanum tuberosum* L.), and sugar beets (*Beta vulgaris* L.), but it is particularly destructive to beans (*Phaseolus vulgaris* L.), soybeans (*Glycine max* (L.) Merr.), pepper (*Capsicum frutescens* L.), and rubber plant (*Ficus elastica* Roxb.), causing considerable losses wherever these crops are cultivated (1,4,6,8,18,19). Isolates of *R. solani* cause root rot and leaf blight on beans and soybeans, root rot on pepper, and leaf blight on rubber plant.

On a worldwide basis, seven anastomosis groups (AG) of *R. solani* have been reported (2,10,12,14). With the exception of AG B1 (10), the anastomosis groups are genetically isolated (3) and differ somewhat from one another in pathological and cultural characteristics (14,17). Although *R. solani* is considered an important problem on a number of crops in Brazil, there are no reports describing anastomosis group relationships and pathogenicity of this fungus in that country other than a short note by Bolkan and Ribeiro (5). The objectives of this study were to determine the anastomosis groups of *R. solani* isolates obtained from Brazil and assess the pathogenicity and optimum temperature requirements within anastomosis groups.

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MATERIALS AND METHODS

Isolates and anastomosis group identification. One hundred thirty isolates were obtained from various geographic regions and host species found in Brazil (Table 1). All isolates were cultured from infected plants or from soil taken from the rhizosphere of diseased plants. Each isolate was hyphal-tipped and conformed to the current species concept of *R. solani* (15). Throughout the study, all isolates were maintained on potato-dextrose agar (PDA) slants at 10 ± 1 C and transferred periodically to fresh medium.

The isolates were subjected to hyphal anastomosis pairing by using the tester isolates (AG-1, AG-2, AG-3, AG-4, and AG-5) supplied by E. E. Butler, Department of Plant Pathology, University of California, Davis. Hyphae plus agar (5 mm in diameter) from tester and unknown isolates were placed 4 cm apart on a sterile microscope slide coated with a thin layer of 2% water agar. Anastomosis was determined microscopically after the

Table 1. Origin and anastomosis groups (AG) of 130 isolates of *Rhizoctonia solani* from Brazil

Host	Source	State	No. of isolates tested	AG
Artichoke	Root	São Paulo	1	4
Barley	Root	Goias	1	4
	Root	Rio Grande do Sul	2	–[a]
Bean	Seeds	Goias, Minas Gerais	10	4
	Hypocotyl	Goias, São Paulo	7	4
	Leaves	Goias, Amazonas	2	1
Bell pepper	Fruit	Federal District	3	4
	Hypocotyl	Amazonas, Rio de Janeiro	2	1,4
Black pepper	Hypocotyl	Para, Minas Gerais	8	1,4
Butterfly pea	Root	Para	1	4
Carrot	Root	Federal District	1	4
Cabbage	Leaves	Amazonas	2	2
Chicory	Root	Rio de Janeiro	1	4
Ciratro	Leaves	Amazonas	1	2
Coffee	Root	São Paulo, Minas Gerais	4	4
Cotton	Hypocotyl	Goias, Parana	3	4
Cowpea	Hypocotyl	Rio Grande do Sul	1	4
Cucumber	Leaves	Amazonas	1	1
Eggplant	Fruit	Federal District	1	4
Fig	Unknown	Pernambuco	1	2
Hibiscus	Root	Rio de Janeiro	1	4
Lettuce	Leaves	Rio de Janeiro	1	1
Orange	Unknown	Federal District	1	1
Potato	Tuber	São Paulo, Goias	10	3
	Stem	Goias	2	4
Peanut	Hypocotyl	Sao Paulo	1	4
Pea	Hypocotyl	Federal District	1	4
Radish	Leaves	Amazonas	2	1
Rice	Stem	Goias, São Paulo	2	4,1
Rubber plant	Leaves	Amazonas, Para, Rondonia	38	2
Sorgum	Root	Rio Grande do Sul	4	–
Soybean	Leaves	Federal District	1	1
	Hypocotyl	Federal District	4	4
Sugar beet	Root	Federal District	1	4
Tea	Leaves	São Paulo	1	4
Triticale	Root	Goias	2	–
	Root	Federal District	1	4
Wheat	Root	Goias	2	4
	Soil	Federal District	2	4

[a] Isolates failed to anastomose with tester isolates representing AGs 1–5.

tester-unknown pairings were incubated for 48–72 hr at room temperature (25 ± 2 C). Each pairing was repeated at least twice.

Pathogenicity tests. Pathogenicity tests of 35 randomly selected isolates of *R. solani* belonging to four anastomosis groups were conducted on six plant species in a greenhouse with temperatures varying from 18 C at night to 30 C during the day. All 35 isolates were tested for pathogenicity to hypocotyls and leaves of kidney beans (cultivar Preto 153) and soybean (cultivar Jupiter); to hypocotyls of radishes (cultivar Redondo Vermelho Gigante), red pepper (cultivar Malagueta), and sugar beet (cultivar Wonder Precoce); and to leaves of cabbage (cultivar Rodondo da Hollanda).

Pathogenicity of the isolates to hypocotyls was tested by growing the plants in soil amended with macerated mycelium. The soil used was a latosol, pH 5.2, sifted through a 2-mm-mesh sieve and steamed for 1 hr at 120 C. Inoculum for infestation of soil was prepared by comminuting a 7-day-old culture on PDA in 100 ml of sterile water for 3 min with a Waring Blendor. Fifteen milliliters of this suspension of mycelium, sclerotia, and agar was thoroughly mixed with 400 g of soil (dry wt equivalent) in plastic pots (13 × 11 × 11 cm). The infested soil was kept moist for 3 days before planting. Ten seeds were planted in each of eight replicate pots of infested soil. Five days after emergence, seedlings were thinned to five per pot. Seedlings grown in soil amended with autoclaved inoculum served as controls. Thirty days after planting, seedlings in each pot were uprooted and washed under running tap water and rated for disease severity on a scale from 0 (no apparent symptoms) to 4 (seedlings killed). Ratings of 1, 2, and 3 refer to 1–25, 26–50, and 51–75% of hypocotyl tissues necrotic, respectively.

Pathogenicity of the isolates to leaves was determined by inoculating leaves of 30-day-old seedlings growing in previously steamed soil in plastic pots (13 × 11 × 11 cm) in a greenhouse or by inoculating 30-day-old detached leaves in plastic boxes as described by Galindo et al (7). Inoculations were made by placing a mycelial-agar disk (6 mm in diameter, taken from a 3-day-old culture on PDA) in the center of each leaf. After inoculations, plants were covered with a plastic bag for 24 hr. Leaves similarly prepared, but that received only PDA disks, served as controls. There were three leaves per replicate and four replicates per treatment. Five days after inoculation, the severity of leaf infection was rated on a scale of 0 (no apparent symptoms) to 4 (more than 75% of leaf surface area necrotic). Ratings of 1, 2, and 3 refer to 1–25, 26–50, and 51–75% of leaf surface area necrotic, respectively.

Vegetative growth at different temperatures. Vegetative growth of the 35 *R. solani* isolates was observed at 10, 15, 20, 25, 28, 30, and 35 C. Mycelial growth was determined in petri plates containing 15 ml of PDA inoculated with mycelium plugs plus agar (4 mm in diameter) cut from a 5-day-old culture of *R. solani* on PDA. Mycelial growth in six replicated cultures at each temperature studied was measured after 72 hr.

RESULTS

Anastomosis group identification. One hundred twenty-two cultures of *R. solani* obtained from 31 plant species found in 11 states in Brazil were separated into four anastomosis groups (Table 1). Eight isolates from three host species found in two states did not anastomose with any of

Table 2. Anastomosis groups (AG) and pathogenicity of 35 isolates of *Rhizoctonia solani* from Brazil on hypocotyl (HP) and/or leaves (LF) of six host plants

Isolate no.	Host	Source	AG	Host tested and disease severity[a]							
				Kidney bean		Red pepper	Soybean		Radish	Sugar beet	Cabbage
				HP	LF	HP	HP	LF	HP	HP	LF
645	Kidney Bean	Leaf	1	1.5	3.5	NP[b]	1.3	NP	NP	1.9	0.7
630	Kidney Bean	Leaf	1	1.7	4.0	1.2	1.4	NP	NP	0.9	NP
617	Black pepper	Hypocotyl	1	NP	4.0	1.1	1.5	NP	0.6	1.0	NP
577	Bell pepper	Hypocotyl	1	NP	2.5	3.5	2.5	1.2	NP	1.5	NP
631	Cucumber	Leaf	1	1.9	4.0	0.6	2.6	NP	NP	1.4	NP
634	Lettuce	Leaf	1	1.2	4.0	1.1	3.1	3.5	0.9	2.6	2.7
298	Orange	Unknown	1	1.3	4.0	2.0	1.3	2.3	2.0	2.9	NP
497	Radish	Leaf	1	3.0	4.0	1.0	3.0	NP	0.7	2.4	1.7
128	Soybean	Leaf	1	1.4	4.0	2.8	2.3	3.8	3.9	2.7	2.0
496	Cabbage	Leaf	2	2.5	NP	0.5	1.5	NP	0.7	3.7	3.7
579	Ciratro	Root	2	NP	2.0	1.0	1.3	NP	NP	0.6	NP
629	Fig	Unknown	2	NP	NP	NP	1.5	NP	NP	2.2	NP
502	Rubber plant	Leaf	2	NP	NP	NP	2.3	NP	NP	2.9	NP
625	Potato	Tuber	3	NP	NP	NP	NP	NP	NP	NP	NP
626	Potato	Tuber	3	NP	NP	NP	NP	NP	NP	NP	NP
627	Potato	Tuber	3	NP	NP	NP	NP	NP	NP	NP	NP
138	Kidney Bean	Hypocotyl	4	2.0	4.0	3.5	3.5	3.8	3.1	4.0	3.3
644	Kidney Bean	Hypocotyl	4	1.0	4.0	0.9	1.3	NP	0.5	1.1	4.0
633	Kidney Bean	Hypocotyl	4	2.6	4.0	1.2	3.9	NP	NP	4.0	NP
47	Kidney Bean	Seed	4	1.2	4.0	2.3	1.9	2.5	2.5	4.0	3.0
317	Bell pepper	Fruit	4	2.5	4.0	3.6	3.6	3.0	3.5	3.1	2.3
618	Butterfly pea	Root	4	NP	4.0	0.8	1.3	2.8	NP	3.3	NP
610	Carrot	Root	4	3.2	4.0	3.9	3.8	1.0	3.8	3.8	4.0
142	Coffee	Root	4	NP	NP	1.1	1.3	0.8	NP	1.0	NP
636	Chicory	Root	4	1.3	4.0	1.6	2.8	4.0	3.0	3.0	2.0
580	Cotton	Hypocotyl	4	2.1	3.8	2.8	4.0	4.0	3.3	3.9	3.7
611	Eggplant	Fruit	4	1.7	4.0	1.0	3.5	3.3	2.2	3.0	2.7
632	Hibiscus	Root	4	1.7	4.0	2.2	3.9	4.0	2.5	3.5	3.0
13	Potato	Stem	4	NP	1.5	0.6	1.5	NP	0.5	1.8	NP
571	Potato	Stem	4	NP	NP	0.5	1.8	NP	NP	1.8	NP
643	Rice	Stem	4	1.4	4.0	0.9	0.9	NP	0.5	1.1	NP
303	Soybean	Hypocotyl	4	1.3	4.0	3.0	4.0	3.3	3.7	3.9	3.7
615	Sugar beet	Hypocotyl	4	2.3	4.0	3.4	3.8	3.5	2.2	3.5	3.0
641	Tea	Leaf	4	NP	NP	0.7	1.2	0.8	0.5	2.7	NP
481		Soil	4	0.9	3.0	1.3	3.0	NP	2.8	3.1	NP

[a] Disease severity was based on a scale of 0 (apparently healthy) to 4 (seedling killed or more than 75% of the leaf surface area affected).
[b] NP = nonpathogenic.

the tester strains or among themselves. Of the 122 isolates, 11 belonged to AG-1, 42 to AG-2, 10 to AG-3, and 59 to AG-4. No isolate was found to belong in AG-5.

AG-2 was the predominant group in the state of Amazonas and was the only group found in association with leaves of rubber plant (Table 1). AG-4 isolates were cultured from 21 plant species and were found in all states surveyed except Amazonas (Table 1). AG-3 isolates were all from sclerotia on potato tubers cultivated in the state of Goias or São Paulo, whereas AG-1 isolates were obtained from leaves or hypocotyls of various crops grown in five states (Table 1).

Pathogenicity tests. Generally, virulence of the *R. solani* isolates tested varied among anastomosis groups, but not within anastomosis groups. All AG-1 isolates tested were highly virulent on kidney bean leaves but were mainly weakly virulent or avirulent on other host plants studied (Table 2). With the exception of one isolate that was highly virulent on cabbage and sugar beet, AG-2 isolates were avirulent or weakly virulent on plants inoculated (Table 2). Most AG-4 isolates were highly virulent on kidney bean leaves and sugar beet hypocotyls, and they had a wider host range than isolates belonging to other anastomosis groups (Table 2). AG-3 isolates were not pathogenic on plant species tested (Table 2).

None of the control seedlings developed symptoms. *R. solani* was reisolated from infected seedlings and compared with the original isolate by studying the nuclear number and morphological characteristics and determining anastomosis group. All reisolates had characteristics similar to the original inoculum.

Vegetative growth at different temperatures. The temperature range for the *R. solani* isolates tested was 15–35 C, with optima at 20–30 C. All 35 isolates grew relatively well at 15 C, whereas 75% of them either did not grow or grew poorly (trace growth) at 10 C. Most isolates showed optimum growth at 30 C, but more than 78% did not grow at 35 C. No significant ($P = 0.05$) variations were observed in temperature optimum requirements between anastomosis groups.

DISCUSSION

Earlier researchers (14) have shown that most isolates of *R. solani* can be separated into four anastomosis groups (i.e., AG-1, AG-2, AG-3, and AG-4). Recently, three more groups (i.e., AG-5, AG-6, and AG B1) have been added along with subdivisions of AG2-1 and AG2-2 groups (10–13). Most Brazilian isolates used in this study were identified to belong to AG-4. Isolates belonging to AG-1, AG-2, and AG-3 were also present, but no isolate was assigned to AG-5. In this study, no attempts were made to separate AG-2 isolates into the two subdivisions.

Most authors agree that hyphal anastomosis groups in *R. solani*, which represent genetic isolation (3), are not host specific although some tendencies are evident (9,14,16,19). Our pathogenicity results are in agreement with these reports. Other than the AG-3 isolates, which were avirulent on all plant species tested, most isolates from different anastomosis groups were capable of causing infection on a variety of plant species. In general, however, isolates within a given anastomosis group were somewhat uniform in their virulence on certain hosts. For example, all AG-1 isolates were highly virulent on kidney bean leaves; weakly to moderately virulent on red pepper, soybean, and sugar beet hypocotyls; but mostly avirulent or weakly virulent on soybean leaves, radish, and cabbage. Generally, AG-4 isolates were more virulent and had a wider host range than isolates belonging to other anastomosis groups. Results of this study further indicate that the AG-2 and AG-3 isolates show a greater host specificity, whereas isolates of AG-1 and AG-4 isolates lack such specificity. Grisham and Anderson (9) observed similar results with AG-1 and AG-4 isolates with which they worked.

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