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Research

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ABSTRACT

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Variability of 45 isolates of *Rhizoctonia solani* (teleomorph *Thanatephorus cucumeris*) causing web blight (WB) of common bean, *Phaseolus vulgaris*, was examined by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis of the internal transcribed spacer regions (ITS1 and ITS2) and the 5.8S subunit (5.8S) of the nuclear ribosomal DNA repeat (ITS-5.8S-rDNA). Isolates were collected from diseased bean leaves from Argentina, Costa Rica, Cuba, Dominican Republic, Honduras, Panama, and Puerto Rico. These WB isolates belong to AG-1 and AG-2 based on anastomosis reaction. Isolates of AG-1 that cause WB were separated into three distinct groups of RFLP patterns from enzymatic digestion of a 740-bp PCR fragment. Microsclerotia-producing isolates (<1 mm) were differentiated from macrosclerotia-producing isolates (5 to 20 mm) based on PCR-RFLP patterns even though they are placed in the same AG1-1B subgroup by anastomosis reaction. WB isolates of AG-2 were separated into two distinct PCR-RFLP groups as previously reported. AG-1 macrosclerotial-producing isolates were the most virulent, whereas isolates of AG-2 were the least virulent. Genetic variability of the WB pathogen may have influenced the failure or success of management practices implemented in the past in Latin America.

Web blight (WB) of common bean (*Phaseolus vulgaris* L.) is caused by aerially dispersed isolates of *Rhizoctonia solani* Kühn (teleomorph *Thanatephorus cucumeris* (Frank) Donk) resulting in defoliation and pod infection which leads to mild to severe yield and seed quality losses (13,15). Presently, the disease is endemic in the bean-growing regions of the Caribbean and Central America, and efforts to control WB have been ineffective and costly. The pathogen spreads by airborne basidiospores, mycelial bridges between plants, rain-splashed sclerotia, infested soil debris, and infected seeds (13,15). Progress in breeding for WB resistance in common bean has been limited due to considerable variability in varietal reaction and lack of understanding of the pathogen variation and disease resistance mechanisms (3,4).

Rhizoctonia solani is a species complex whose current classification is based on the occurrence of hyphal fusion with known tester isolates to establish an anastomosis group (AG). Currently, 13 AGs have been described with considerable variability existing among and within AGs (7). These variants are described as subgroups and differ in morphology, ecological habitat, survival, and virulence (22,28). At least three AGs (AG-1, AG-2, and AG-4) have been associated with WB of common bean in the Americas (6,13). Based on cultural characteristics and epidemiological differences, isolates of AG-1 and AG-2 of the WB pathogen appear to have intragroup variation (15).

The advent of molecular techniques has provided tools to evaluate intra- and interspecific variation of *R. solani* complex (9). Among these tools, polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) of the internal transcribed spacer (ITS) region of the nuclear rDNA repeat have been used to provide more understanding of the genetic diversity within the *R. solani* complex (9,32). The existence of more genetically distinct subgroups within AG-1, AG-2, and AG-4 (5,18–20,22,28) has also been demonstrated. Understanding the extent of diversity of the WB pathogen could explain variation in the host-pathogen interaction in terms of cultivar reaction and the limited success a decade ago of disease management recommended for web blight of common beans in the tropics. Most *R. solani* research on ecology, epidemiology, and genetics has been conducted with soil-borne isolates causing root rot and stem rots in crops from temperate zones. Aerially dispersed *R. solani* groups have not received much attention even though these isolates cause severe diseases of the aboveground plant parts of economically important crops such as rice, corn, bean, sorghum, and tobacco in the tropics and subtropics (30).

To test the hypothesis that the bean WB pathogen population of Central and South America and the Caribbean was variable, the genetic diversity of WB field isolates was examined using PCR-RFLP of the internal transcribed spacer regions (ITS1 and ITS2) and the 5.8S subunit (5.8S) of the nuclear ribosomal DNA repeat (ITS-5.8S-rDNA) and the virulence of these isolates was determined in a detached leaf test.

MATERIALS AND METHODS

Source of isolates and maintenance. The *R. solani* isolates used in this work were obtained from leaves of commercial cultivars of *P. vulgaris* and wild beans (*P. vulgaris aborigineus*) with symptoms of web blight. These isolates have been assigned to AG-1 and AG-2 in previous studies (14). All available testers were not used to identify *R. solani* subgroups since PCR-RFLP techniques can now be used. Only two testers, R-131 (AG-1-IC, formerly R43 Parmeter's collection) and R-135 (AG-2-2 from Ogoshi's collection; Fig. 1C), representing the AG groups were included for comparison (Table 1). WB isolates of AG-1 were described as microsclerotia-producing with sclerotia size of <1 mm in diameter, macrosclerotia-producing with sclerotia size of 5 to 20 mm in diameter, and "sasakii" sclerotia type (Fig. 1B). Cultures of these isolates are maintained in repositories at the University of Nebraska, Lincoln, and the Health Sciences University of Hokkaido, Japan. All isolates were maintained on sterile sugar beet seeds at 4°C before transferring them to water agar (Difco Agar;

Difco Laboratories, Detroit, MI) and potato dextrose agar (PDA; Difco Laboratories) for growth.

Isolation of mycelia DNA. Isolates were grown on a liquid V8 medium in 22-mm deep, sterile, petri dishes at 24°C for a week. Mycelium was harvested by filtration, lyophilized, and ground to powder for preservation at –5°C in a desiccant. Total genomic DNA was extracted following the procedure of Skroch and Nienhuis (27). The DNA was extracted from 25 mg of powdered, lyophilized mycelium. The samples were incubated for 1 h at 65°C in 500 µl of extraction buffer (100 g potassium ethylxanthagenate, 81.1 g NaCl, 200 ml of 1 M Tris Buffer (pH 7.5), and 40 ml of 0.5 M EDTA per liter of distilled water). After incubation, each sample was centrifuged for 10 min at 14,000 rpm. The supernatant was precipitated with a 6:10 (vol/vol) mixture of ethanol and 7 M ammonium acetate at –20°C. After a 30 s spin, the samples were washed with 70% ethanol, reprecipitated, and then centrifuged again. The pellet was rehydrated in 200 µl of TE buffer (1 mM Tris, 0.1 mM EDTA), and 20 µl of 100 µg/liter of RNase A were added. The suspension was incubated 1 h at 37°C. The DNA was precipitated by adding 500 µl of a 20:1 (vol/vol) mixture of ethanol and 3 M sodium acetate at –20°C. The final dilution was stored in 1× TE buffer. The concentration of each sample was estimated using an Ultrospect III spectrophotometer (Pharmacia LKB Biochrom, Cambridge, England) and diluted to give a final concentration of approximately 10 ng of DNA per µl of buffer.

PCR and RFLP analysis of the rDNA-ITS region. Amplification of the nuclear rDNA region and the ITS regions including the 5.8S ribosomal DNA (ITS-5.8S-rDNA) was performed using primers ITS1 and ITS4 (34). The PCR amplification reactions were performed by adding 1 µl of 500-fold dilution of genomic DNA (10 ng/µl) solution to 50 µl of reaction mixture of *Taq* buffer (500 mM KCL, 100 mM Tris-HCl, pH 8.3, 1.5 mM MgCl$_2$, and 0.001% gelatin) and 100 mM dNTP, 20 mM primers ITS1/ITS4 and two units of *Taq* polymerase (U/ml) (Promega, Madison, WI). Amplification was performed in a GeneE Thermal Cycler with heated lid (Techne Inc., Princeton, NJ) programmed for 40 cycles with the following temperature profile: 1 min at 94°C; 1 min at 53°C; and 105 s at 72°C. Cycling ended with a final extension step at 72°C for 5 min. Amplification products were separated in a 1% Agarose Low EEO (Fisher Scientific Co, Pittsburg, PA) gel in Tris-borate EDTA buffer at 105 V for 1 h and visualized by ethidium bromide staining on a UV transilluminator. Restriction endonuclease digestion of DNA amplification of each isolate was performed with *Msp*I, *Hae*III, *Alu*I, *Mbo*I, and *Taq*I according to Kanamatsu and Naito (18) and Liu and Sinclair (20). *Bsr*DI was included for its usefulness in

Fig. 1. Differences in *Rhizoctonia solani* isolates. **A,** lesion size on common bean cv. HT-7719 of isolates (left) P30 (macrosclerotia-producing AG-1-1B) and (right) A23 (AG-2). **B,** Characteristics of AG-1 isolates on potato dextrose agar (PDA) medium. Top left: sasaki isolate; top right: macrosclerotia-producing isolate; bottom: microsclerotia-producing isolate. **C,** Characteristics of AG-2 isolates on PDA medium. Top row (left to right): R-135, DR-EEAL, and A-1. Bottom row: A18, A19, and A24. **D,** Typical web blight symptoms on leaves and pods of common bean produced by AG-1 isolates. **E,** Web blight symptoms on leaves and pods of common beans produced by AG-2 isolates.

Table 1. Isolates of *Rhizoctonia solani* used in this study

Isolate code	Host	Origin
A1, A4, A6-8, A11	*Phaseolus vulgaris aborigineus*	Jujui Province, Argentina
A18-21, A23, A24, A27, A28	*P. vulgaris aborigineus*	Salta Province, Argentina
H-32-34, H38, H39	*P. vulgaris* cv. Dorado	El Barro, Honduras
H-45, H46	*P. vulgaris* cv. Dorado	Lavanderos, Honduras
CR10, CR11	*P. vulgaris* cv. MUS 181	Guatuso, Costa Rica
DR-BV1, DR-BV18, 22, 33, 42, 98, DR-EEAL, DR-J2, DR-HPD	*P. vulgaris* cv. PC-50	San Juan Valley, Dominican Republic
Cuba 2	*P. vulgaris* unknown	La Habana, Cuba
P6, P10, P30, P35, P40, P41	*P. vulgaris* cv. Barriles	Caizan, Panama
PR5, PR11, PR25, PR34, PR45, PR50	*P. vulgaris* cv. Arroyo Loro	La Isabela, Puerto Rico
R131	…	Formerly R43 Parmeter's collection (AG-1-IC)
R135	…	Ogoshi's collection (AG-2-2)

detecting polymorphisms in other foliar pathogens (24).

Virulence test. Seventeen *R. solani* isolates representing the range of restriction site polymorphisms of the amplified ITS region were tested for virulence. Mycelial disks (20 mm) from 2-day-old *R. solani* cultures grown on PDA were placed on the top surface of a detached second trifoliolate of the bean cv. HT 7719 (WB field tolerant, Middle American origin), placed in an aluminum roasting pan, covered with plastic film which maintains the chamber (pan) at constant high humidity. Leaf lesion area was determined by hand measurement after 48 h at 22°C. This method has been used to screen soybeans, beans, and canola against *Sclerotinia sclerotiorum* (29), a necotrophic pathogen like *R. solani.* The method is currently used for web blight resistance screening (J. Beaver, *personal communication).* The experiment was a randomized complete block design with three replications and was repeated twice. Analysis of variance and mean separation were performed using the MSTAT-C v 2.11 program (Department of Soils and Crops, Michigan State University, East Lansing).

RESULTS

PCR amplification and RFLP of the rDNA-ITS region. Amplification of the ITS-5.8S-rDNA region of the nuclear rDNA repeat of 47 *R. solani* isolates yielded DNA fragments estimated at 740 bp, except tester R-131, which had a 700-bp length. Restriction enzyme digestions of the amplified DNA of all isolates with *Alu*I, *Bsr*DI, *Eco*RI, *Hae*III, *Mbo*I, *Msp*I and *Taq*I restriction enzymes resulted in six distinct patterns (Tables 2 and 3).

AG-1 WB isolates were separated into three distinct RFLP patterns (I to III) from digestions with *Hae*III, *Bsr*DI, *Alu*I, *Mbo*I and *Taq*I (Table 2). The separation of isolates based on these patterns was in agreement with the subgroups separated on morphological and cultural differences. The tester isolate, R-131, gave a pattern that was different from the other AG-1 isolates. The RFLP pattern I was represented by isolate CUBA 2. Restriction enzyme digestions with *Alu*I generated two products consistent with a single restriction site. *Hae*III and *Taq*I restriction enzyme digestions generated three products consistent with two restriction sites. *Mbo*I digestions revealed five restriction sites in the

ITS-5.8S-rDNA region. The restriction enzyme *Bsr*DI yielded only a single fragment of the same size as the amplified region. The restriction enzyme fragments from *Hae*III, *Alu*I, *Taq*I, and *Mbo*I digestions closely resembled those reported by Schneider et al (26) and Liu and Sinclair (20) for AG-1-IA isolates.

The RFLP pattern II was generated by the AG-1-IB microsclerotia-producing isolates of the WB pathogen from the Dominican Republic and Honduras. The amplified ITS-5.8S-rDNA region of these isolates had two restriction sites for *Hae*III, three for *Alu*I (Fig. 2A) and *Taq*I, and four for *Mbo*I. The restriction enzyme *Bsr*DI yielded only a single fragment of the same size as the amplified region (Fig. 2B). Fragments generated by *Taq*I digestions with these isolates were different in size and number from other AG-1-I B isolates from soil and sugar beet and examined by Schneider et al. (26) and Liu and Sinclair (20).

The RFLP pattern III was generated by the macrosclerotia-producing isolates of AG-1-IB from Puerto Rico and Panama. The ITS-5.8S-rDNA region of these isolates had two restriction sites for *Hae*III, *Alu*I (Fig. 2A), and *Taq*I and four sites for

Table 2. Estimated restriction fragment length of the ITS-rDNA region for *Rhizoctonia solani* AG-1 isolates of the web blight pathogen of common beans

| Anastomosis group/subgroups | Fragment length in base pair using restriction enzymes | | | | | ITS-RFLP pattern[y] | Isolates |
	*Hae*III	*Bsr*DI	*Alu*I	*Taq*I	*Mbo*I		
AG-1-IA	550	740	520	380	250	I	Cuba 2
	125		220	300	210		
	65			60	140		
					65		
					50		
					25[z]		
AG-1-IB (microsclerotia-producing)	550	740	340	360	280	II	DR-BV1, DR-BV8, DR-BV22, DR-BV33, DR-BV42,
	125		200	260	250		DR-BV98, H32, H33, H34, H38, H39
	65		120	65	140		
			80	55	55		
					15[z]		
AG-1-IB (macrosclerotia-producing)	550	575	500	380	260	III	PR5, PR11, PR25, PR34, PR45, PR50, P6, P10, P30,
	125	165	200	300	250		P35, P40, P41
	65		40	60	140		
					55		
					35		
AG-1-IC	400	700	500	360	250	IV	R-131
	125		200	280	240		
	100			60	135		
	75				55		

[y] ITS = internal transcribed spacer region. RFLP = restriction fragment length polymorphism.
[z] Fragments 25 bp or smaller in length not visualized on RFLP gel were inferred from nucleotide base pair length.

Table 3. Estimated restriction fragment length of the ITS-rDNA region of *Rhizoctonia solani* AG-2 isolates of the web blight pathogen of common beans

| Anastomosis group/subgroup | Fragment length in base pair using restriction enzymes | | | | ITS RFLP pattern[z] | Isolates |
	*Eco*RI	*Msp*I	*Hae*III	*Taq*I		
AG-2-2	390	590	600	360	V	R-135, DR-EEAL, DR-J2, DR-HPD, CR10, CR11,
	350	150	140	310		A1, A4, A6, A7, A11, H-45, H46
				70		
AG-2 unknown	390	590	400	360	VI	A8, A18, A19, A20, A21, A23, A24, A27, A28
	350	150	190	310		
			140	70		

[z] ITS = Internal transcribed spacer region. RFLP = restriction fragment length polymorphism.

*Mbo*I. Digestions with *Bsr*DI yielded two fragments of 575 and 165 bp (Fig. 2B). Other researchers indicated that *Alu*I and *Mbo*I digestions of the ITS-5.8S-rDNA region showed two and three restriction sites, respectively, for AG-1-I B isolates (20,26).

The RFLP pattern IV was represented by tester isolate R-131 where restriction enzymes *Alu*I, *Taq*I, *Hae*III, and *Mbo*I had one, two, three, and four restriction sites, respectively, in the amplified ITS-5.8S-rDNA region. Restriction enzyme *Bsr*DI yielded only a fragment of the same size as the amplified region (Fig. 2B). These results are in agreement with other reports for AG-1-IC isolates (20,26).

AG-2 WB isolates produced RFLP patterns V and VI from restriction enzyme digestion of the amplified ITS-5.8S-rDNA region (Table 3). These two patterns represented WB field isolates from Costa Rica, Dominican Republic, and Honduras and isolates causing WB in wild beans in the Andean mountain rainforests of Argentina. RFLP pattern V had one restriction site for *Eco*RI, *Msp*I, *Hae*III, and two for *Taq*I. The isolates fit the RFLP pattern previously reported for cultural types AG-2-2 IV from sugar beets (16,18). The RFLP pattern VI showed similar results with *Eco*RI, *Msp*I, and *Taq*I but not with *Hae*III, which showed two restriction sites (Fig. 3) (16). A Japanese AG-2-1 isolate from peas and isolates of a newly described subgroup AG-2t, associated with diseases of crucifers and tulips in the Netherlands, have two restriction sites in the ITS-5.8S-rDNA region (26). However, they can be clearly differentiated from the AG-2 isolates that cause WB on wild beans by fragment lengths in the RFLP profile.

Virulence. All of the isolates caused lesions on the second trifoliolates of bean plants (Table 4). Isolates typed to AG-1 developed extensive water-soaked and necrotic lesions within 2 days after inoculation and were collectively more virulent than those of AG-2 (Fig. 1A). Macrosclerotia-producing isolates were more virulent than the microsclerotia-producing isolates of the AG-1-IB subgroup on the bean

genotype tested. Visible lesions produced by AG-2 isolates developed a day later than those produced by AG-1, and these AG-2 caused-lesions were not accompanied by water soaking but did produce yellowing and further necrosis. Isolate A28, collected from wild beans in Argentina, was significantly more virulent than the other AG-2 isolates.

DISCUSSION

Web blight of common bean is caused by at least five genetically distinct *R. solani* subgroups based on the polymorphism of restriction enzyme digestions of the ITS-5.8S-rDNA region of 45 isolates. The WB isolates were obtained from bean-growing regions in seven countries in Latin America and the Caribbean and those classified as AG-1 fell into three distinct ITS-RFLP patterns while those classified as AG-2 showed two patterns. In our study, isolates from AG-1 or AG-2 that shared similarities in cultural characteristics, sclerotia, and virulence, also displayed similar RFLP patterns regardless of geographical or varietal host origin. Traditionally, AG-1 isolates of *R. solani* have been subdivided into AG-1-IA, AG-1-IB, and AG-1-IC according to host origin, symptoms, sclerotia form, cultural characteristics, and DNA sequence homology (22,28). The AG-1-IA (rice sheath blight) isolates are known for their "sasakii" (primitive rind) sclerotia type and cause sheath blight of rice, sorghum, and corn and aerial blights of soybean (17,28,35). The AG-1-IB is the web blight form of *R. solani* AG-1 and affects various row crops and ornamentals in tropical and subtropical regions (Fig. 1D). The AG-1-IC (damping-off) isolates affect primarily seedlings of various crops (22,28). Only one isolate from a black seeded bean variety grown in northern Cuba was separated by RFLP banding pattern and cultural characteristics into subgroup AG-1-IA. The remaining isolates were assigned to AG-1-IB based on molecular differentiation of AG-1 isolates by Liu and Sinclair (20).

The ITS-RFLP method also was able to discriminate isolates that produce micro- or

macrosclerotia in culture, as separate genetic entities. Both had been previously assigned to AG-1-IB based on subgroup description even though cultural and morphological characteristics warranted their separation (23). Other researchers have reported that isolates causing web blight of common bean and aerial blight of soybean produce micro- and macrosclerotia simultaneously on host tissue (2,31,33). Our isolates however, showed only one distinctive sclerotia type on PDA and only microsclerotia-producing isolates have developed sclerotia under field conditions (*unpublished data*). These microsclerotia can function as airborne propagules causing secondary infections and facilitating pathogen spread and inoculum build up (35).

Microsclerotia- and macrosclerotia-producing isolate banding patterns consistently showed evidence of genetic homogeneity within both subgroups. We did not
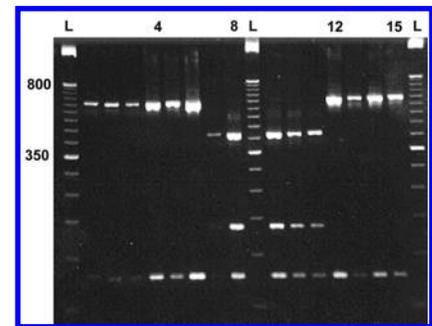
Fig. 3. Restriction digestion patterns of the nuclear ribosomal DNA-internal transcribed spacer region between primers ITS1 and ITS4 in *Rhizoctonia solani* AG-2 isolates using *Hae*III endonuclease. L: 50-bp ladder; 1: R135 (AG2-2 tester); 2: A1; 3: A4; 4: A6; 5: A7; 6: A11; 7: A18; 8: A20; 9: A21; 10: A23; 11: A28; 12: DR-J2; 13: DR-HPD; 14: CR-10; 15: CR-11.

Table 4. Lesion area produced on trifoliolate leaves of common bean cv. HT7719 in humid chambers by *Rhizoctonia solani* that causes web blight

Isolate	Lesion area (cm²)
PR5	5.12 a[z]
P30	3.89 b
P6	3.85 b
Cuba 2	3.20 c
R131	3.06 c
A28	3.02 cd
H32	3.01 cd
DR-BV22	2.93 cde
DR-BV1	2.57 cde
A27	1.81 defg
DR-EEAL	1.73 fg
A23	1.43 fg
A4	1.33 g
H46	1.04 g
DR-J2	0.89 g
H45	0.87 g
R135	0.77 g
LSD (*P* = 0.05)	1.23

[z] Values in column followed by the same letter are not significantly different at *P* = 0.05 according to Fisher's least significant difference test transformed data.
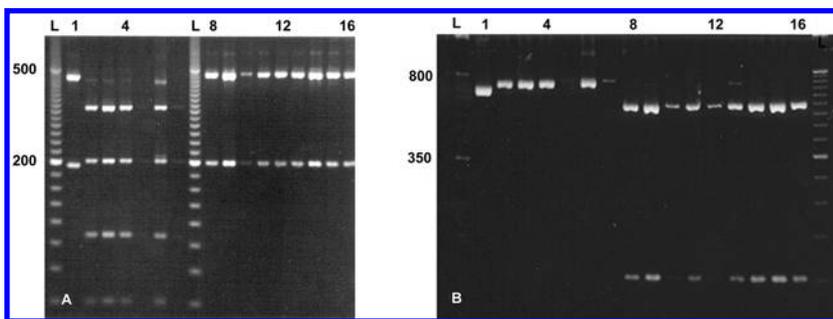
Fig. 2. Restriction digestion patterns of the nuclear ribosomal DNA-internal transcribed spacer region between primers ITS1 and ITS4 in *Rhizoctonia solani* AG-1 isolates using *Alu*I and *Bsr*DI endonucleases. **A,** L: 20-bp ladder (*Alu*I) and **B,** L: 50-bp ladder (*Bsr*DI); 1: R131 (AG-1-IC tester); 2: BV1; 3: BV22; 4: BV36; 5: H32; 6: H33; 7: H34; 8: PR5; 9: PR11; 10: PR25; 11: PR34; 12: PR45; 13: PR50; 14: P6; 15: P10; 16: P30.

detect any heterogeneity or random variation in ITS-RFLP profiles from any isolate as previously reported for isolates within subgroups of AG-1 and AG-2 (16,26). Liu and Sinclair (20) characterized isolates that did not conform to AG-1 subgroups IA, IB, or IC as ID, IE, and IF using the rDNA-ITS and isozyme profiles. Our isolates did not fully conform to these profiles. The ITS-RFLP profiles suggest a further separation of AG-1-IB isolates of the WB pathogen of common beans that produce microsclerotia from those that produce macrosclerotia. Work is in progress to sequence the amplified rDNA-ITS region to obtain more genetic information on these isolates. Web blight symptoms on common bean also have been associated with undescribed subgroups of AG-2 (6,11). However, these symptoms more closely fit the description of foliar blights than typical web blights since AG-2 isolates do not develop "cobweb-like mycelia" on foliage. Instead, infection-causing basidiospores are produced and disseminated from hymenia borne underneath infected trifoliolate leaves. Small (1 mm) circular necrotic lesions are observed commonly in the upper plant canopy (Fig. 1E). When conditions are humid and warm, these lesions develop secondary, irregularly shaped, water-soaked lesions that expand and coalesce giving a "scalded" appearance to infected plants. The basidial stage of the WB pathogen has been reported in bean fields in Costa Rica, Colombia, and Dominican Republic (6,10,14). Aerial isolates of *R. solani* AG-2 incite foliar blights of economically important crops such as sugar beet and soybean (21,22) in Japan and the United States.

AG-2 isolates can be identified according to hyphal fusion frequency, thiamine requirements, and cultural characteristics (28). However, these are time consuming and require considerable expertise. Molecular techniques, such as PCR-RFLP and rDNA sequence analysis, have facilitated the identification of cultural and ecological types within subgroups. Currently, there are three subgroups: AG-2-1, AG-2-2 (with three cultural types IIIB, IV, and LP), and AG-2-3. A newly defined cultural type AG-2t also has been differentiated on the basis of sequences of the ITS- rDNA region (26).

Interestingly, our isolates from bean fields in Costa Rica, Dominican Republic, and Honduras as well as some rainforest isolates from argentinian wild bean were assigned to AG-2-2 IV. Members of this cultural type have been previously described as pathogens to Chenopodiaceae hosts such as sugar and table beets (22,28). This indicates that host range and geographical location are becoming less important in delimiting subgroups of *R. solani*. The finding that ITS-RFLP patterns of wild bean isolates differ from those of previously described subgroups placed into the AG-2 category demonstrates the need to obtain more information on indigenous plant pathogens at the center of host origin.

Subgroups of *R. solani* AG-2 are characterized as genetically stable independent populations and exist in very broad geographical regions (19). Regional germ plasm exchange of unknowingly contaminated seed of land races or wild beans could be one important avenue for widespread distribution of undescribed subgroups of *R. solani*.

Variation in virulence also was recorded among subgroups of the WB pathogen. AG-1 macrosclerotia-producing isolates were the most virulent, whereas isolates of AG-2 were the least virulent. Similar results have been reported with Puerto Rico WB-isolates in greenhouse virulence tests (23) and in field trials of the Latin American and Caribbean Nurseries where virulence patterns varied among countries of origin and bean genotypes (3).

The observation of a wide range of virulence on HT 7719 has important implications for breeding for WB resistance. This bean line was developed specifically for web blight resistance in 1986 by the Centro Internacional de Agricultura Tropical (CIAT) and has been widely used as a parent in breeding programs in Latin America (8). Over time, development and spread of virulent fungal pathotypes or new subgroups of *R. solani* could have overcome resistance in HT 7719.

In *R. solani*, genetic variation in populations can be due to sexual recombination, heterokariosis fusion, and mutation (1). Genetic analysis of populations of *R. solani* AG-1-IA, causal agent of sheath blight of rice in Texas, revealed that novel genotypes were generated by sexual recombination and increased by asexual reproduction to high frequency (25). Similar mechanisms could allow AG-1 isolates of the WB pathogen to develop new virulent types since these isolates can develop the sexual stage under favorable field conditions (2,12,33,36). Spatial and temporal distribution of *R. solani* pathotypes can occur via asymptomatic infected seed (15) and other agronomic practices that contribute to dispersal of asexual propagules such as mycelia and sclerotia.

Knowledge about the genetic composition of aerial *R. solani* populations have influenced management decisions in other crops such as soybean and rice where crop rotation, intercropping, breeding, and cultural and chemical control have been modified and/or improved based on proper identification of the predominant subgroup (17). For common bean, the finding of different subgroups associated with WB will influence management practices implemented for this disease in the tropics.

ACKNOWLEDGMENTS

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