Virulence and Molecular Diversity in *Colletotrichum lindemuthianum* from South, Central, and North America

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ABSTRACT

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Isolates of *Colletotrichum lindemuthianum* (138 total) from Argentina, Brazil, the Dominican Republic, Honduras, Mexico, and the United States were characterized into 41 races based on virulence to 12 differential cultivars of *Phaseolus vulgaris*. These 41 races were categorized into two groups: those found over a wide geographic area and those restricted to a single country. Races 7, 65, and 73 were widespread. Race 73 was the most common (28%). Race 7 was found once in Argentina and Mexico but at a higher frequency in the United States. Race 65 was found repeatedly in Brazil and the United States. Although 39% of the races were

detected repeatedly and three races were widespread, no race was isolated from both *P. vulgaris* gene pools. Phenetic analyses showed no obvious patterns correlated with virulence clusters. No geographic pattern was evident. Molecular polymorphism generated by random amplified polymorphic DNA confirmed the extensive variability in virulence of *C. lindemuthianum*. Virulence phenotypes were grouped into 15 clusters. The two largest clusters contained isolates from all the geographic regions sampled. Molecular polymorphism was observed among isolates from races 65 and 73 within and among countries, except among Brazilian isolates of race 65. The genetic diversity of *C. lindemuthianum* was greatest in Mexico and Honduras. Our data suggest that *C. lindemuthianum* may not be highly structured to specific *Phaseolus* gene pools.

Colletotrichum lindemuthianum (Sacc. & Magnus) Lams.-Scrib. causes anthracnose of common bean (*Phaseolus vulgaris* L.). The disease is found worldwide wherever common beans are grown, but is especially important in subtropical and temperate regions (32). Yield losses can reach or exceed 90% during some years (48). Because cost-effective chemical control is lacking, host resistance traditionally has been used to control anthracnose, despite high variability in the virulence of *C. lindemuthianum* (1,4-6,13, 19,23,43). A standardized binary nomenclature system based on a set of 12 differential cultivars has been developed to characterize virulence (28). Using this system, 38 races were reported in Mexico (14,35), 7 races were identified in a group of 10 isolates from Nicaragua (33), 33 races were characterized from a group of 178 isolates from Colombia (31), and 3 races were described in the United States (2,18). Because only a few races are extremely widespread, it is unknown whether the virulence pattern of *C. lindemuthianum* is due to repeated evolution of a race or efficient seedborne dispersal between areas (32).

Virulence in the common bean pathogens *Uromyces appendiculatus* (21) and *Phaeoisariopsis griseola* (17) appears to be highly structured within each species. Patterns generated by randomly amplified polymorphic (RAPD) DNA markers indicated that *U. appendiculatus* was highly structured geographically (21). RAPD patterns divided *Phaeoisariopsis griseola* isolates into two distinct groups: one group was specialized on *P. vulgaris* hosts of Andean ancestry, and the second group was obtained from common bean hosts of Middle American origin (15,38). This grouping of fungal races congruently with host gene pools has been interpreted as

evidence for coevolution (17,29,38). It also suggests combining complementary genes to exclude all pathogen lineages (7,10,20, 46) where resistance genes from Middle American hosts can be utilized effectively in Andean hosts and vice versa. Indeed, virulence within common bean rust (*U. phaseoli*) is specialized on either Andean or Middle American germ plasm (21,40,41), leading some authors to advocate lineage-based breeding to control this (15) and other common bean pathogens, including *C. lindemuthianum* (38,46).

A study that utilized RAPD markers and restriction fragment length polymorphism (RFLP) of both ribosomal genes and the internal transcribed region of the ribosomal genes found that *C. lindemuthianum* isolates consistently formed two main groups, but some isolates did not fall distinctly into one group or the other (12). The limited number of isolates used did not allow analysis of whether groupings were aligned according to host of origin or virulence. A similar study conducted with isolates of *C. lindemuthianum* collected from a wild *Phaseolus* host in Mexico, Ecuador, and Argentina showed groupings of isolates according to the Andean and Middle American host gene pools (37). In this study we sought to determine how variability within *C. lindemuthianum* is structured. Isolates were collected from both Andean and Middle American *Phaseolus* genotypes grown in Argentina, Brazil, Canada, the Dominican Republic, Honduras, Mexico, and the United States. Virulence analyses and RAPD polymorphism were used separately to group isolates by cluster analyses.

MATERIALS AND METHODS

***C. lindemuthianum* isolates.** Conidial isolates (138 total) were collected between 1992 and 1994 from common bean cultivars grown in Argentina, Brazil, the Dominican Republic, Honduras, Mexico, and the United States (Table 1). The fungus was isolated from diseased leaves or pods showing characteristic anthracnose

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symptoms (32). Small pieces of infected tissue were surface-sterilized and incubated on petri dishes containing modified Mathur's culture medium. The culture medium was prepared with dextrose (8 g liter⁻¹), MgSO₄·7H₂O (2.5 g liter⁻¹), KH₂PO₄ (2.7 g liter⁻¹), neopeptone (2.4 g liter⁻¹), yeast extract (2.0 g liter⁻¹), and agar (16 g liter⁻¹). Plant tissue was incubated in complete darkness at 24°C for 7 days or until formation of acervuli morphologically resembling *C. lindemuthianum* (32). Spore suspensions for seedling inoculation were prepared from purified single-conidial isolates by flooding plates with 5 ml of 0.01% Tween 80 in distilled water. Spores were dislodged by scraping the culture surface with a spatula and filtered through cheesecloth, and the concentration was adjusted to 1.0 × 10⁶ spores per ml with a hemacytometer.

Determination of virulence phenotypes. Races of *C. lindemuthianum* were characterized based on the virulence of the 138 isolates on the differential *P. vulgaris* series proposed by Pastor-Corrales (28). Seeds for each of the 12 cultivars were planted in flats containing Baccto planting mix (Michigan Peat Co., Houston) and grown under greenhouse conditions (16-h day at 25°C) for 7 to 10 days until seedlings had reached the primary leaf stage. Seedlings (6 to 10) were spray-inoculated with standardized spore suspensions of each isolate of *C. lindemuthianum*. Suspensions were applied until runoff on the stem and to both surfaces of the unifoliolate leaves. After inoculation, plants were maintained in high humidity (>95%) for 48 h at 22 to 25°C. Plants were allowed to dry and transferred to greenhouse benches for 5 days before disease symptom evaluation. Seven days after inoculation, seed-

TABLE 1. Race designation, number of isolates, and susceptible (+) and resistant (–) reactions of common bean differential cultivars to 41 races of *C. lindemuthianum* identified from 138 isolates collected on *Phaseolus* hosts from Middle American and Andean gene pools grown in the countries listed

Race ^a	Differential cultivars and known resistance genes ^b												Isolates ^c
	A	B ^d Co-1	C ^d	D Co-2	E	F ^d	G Co-3	H	I Co-4	J Co-5	K Co-6	L Co-4 ² /Co-5/Co-7	
1	+	–	–	–	–	–	–	–	–	–	–	–	Bra (M-3)*
2	–	+	–	–	–	–	–	–	–	–	–	–	Mex (A-1)
7	+	+	+	–	–	–	–	–	–	–	–	–	Arg (A-1), Mex (A-1), US (A-6)*
9	+	–	–	+	–	–	–	–	–	–	–	–	Hon (M-4)*
17	+	–	–	–	+	–	–	–	–	–	–	–	Bra (M-5)*
19	+	+	–	–	+	–	–	–	–	–	–	–	DR (A-1)
55	+	+	+	–	+	+	–	–	–	–	–	–	DR (A-1)*
65	+	–	–	–	–	–	+	–	–	–	–	–	Bra (M-6)*, US (M-13)*
73	+	–	–	+	–	–	+	–	–	–	–	–	Bra (M-3)*, Hon (M-4)*, Mex (M-2)*, US (M-29)*
81	+	–	–	–	+	–	+	–	–	–	–	–	Arg (M-1)
89	+	–	–	+	+	–	+	–	–	–	–	–	Bra (M-2)*
193	+	–	–	–	–	–	+	+	–	–	–	–	Mex (M-1)
201	+	–	–	+	–	–	+	+	–	–	–	–	Hon (M-1)*
209	+	–	–	–	+	–	+	+	–	–	–	–	Mex (M-1)
256	–	–	–	–	–	–	–	–	+	–	–	–	Mex (M-1)*
257	+	–	–	–	–	–	–	–	+	–	–	–	Mex (M-1)*
320	–	–	–	–	–	–	+	–	+	–	–	–	Mex (M-1)
321	+	–	–	–	–	–	+	–	+	–	–	–	Mex (M-3)*
337	+	–	–	–	+	–	+	–	+	–	–	–	Bra (M-1)
357	+	–	+	–	–	+	+	–	+	–	–	–	Mex (M-3)*
448	–	–	–	–	–	–	+	+	+	–	–	–	Mex (M-5)
449	+	–	–	–	–	–	+	+	+	–	–	–	Mex (M-9)*
453	+	–	+	–	–	–	+	+	+	–	–	–	Mex (A-2)*
457	+	–	–	+	–	–	+	+	+	–	–	–	Mex (M-4)*
465	+	–	–	–	+	–	+	+	+	–	–	–	Mex (M-1)
469	+	–	+	–	+	–	+	+	+	–	–	–	Mex (M-1)
521	+	–	–	+	–	–	–	–	–	+	–	–	Hon (M-1)
833	+	–	–	–	–	–	+	–	+	+	–	–	Mex (e-1)
1033	+	–	–	+	–	–	–	–	–	–	+	–	Hon (M-1)*
1165	+	–	+	+	–	–	–	+	–	–	+	–	Mex (e-1)*
1344	–	–	–	–	–	–	+	–	+	–	+	–	Mex (M-1)
1431	+	+	+	–	+	–	–	+	+	–	+	–	Mex (M-1)
1472	–	–	–	–	–	–	+	+	+	–	+	–	Mex (M-2)*
1545	+	–	–	+	–	–	–	–	–	+	+	–	Hon (M-1)
1600	–	–	–	–	–	–	+	–	–	+	+	–	Mex (M-1)
1601	+	–	–	–	–	–	+	–	–	+	+	–	Hon (M-1)
1673	+	–	–	+	–	–	–	+	–	+	+	–	Hon (M-2)*
1677	+	–	+	+	–	–	–	+	–	+	+	–	Hon (M-1)
1741	+	–	+	+	–	–	+	+	–	+	+	–	Hon (M-1)*
1929	+	–	–	+	–	–	–	+	+	+	+	–	Hon (M-1)*
1993	+	–	–	+	–	–	+	+	+	+	+	–	Hon (M-4)*

^a Race identification according to the binary nomenclature system (28). Races previously designated by Greek letters: 17, α; 55, λ; 65, ε; 81, μ; 89, α-Brazil; and 453, ζ.

^b Common bean differential cultivars used to characterize races of *C. lindemuthianum* followed by their respective binary value (28): A: Michelite, 1; B: Michigan Dark Red Kidney, 2; C: Perry Marrow, 4; D: Cornell 49242, 8; E: Widusa, 16; F: Kaboon, 32; G: Mexico 222, 64; H: PI 207262, 128; I: TO, 256; J: TU, 512; K: AB 136, 1024; and L: G 2333, 2047. Known *Co* resistance genes are shown (45,47).

^c Geographic origin of isolates: Arg = Argentina; Bra = Brazil; DR = Dominican Republic; Hon = Honduras; Mex = Mexico; and US = United States, followed in the parentheses by the host gene pool (A = Andean and M = Middle America) and the number of times the race was isolated in the country. The gene pool was determined based on host genotype identified during sampling of isolates and supported by previous knowledge. * indicates isolate characterized by randomly amplified polymorphic DNA.

^d Cultivars from the Andean gene pool.

^e Host genotype not identified.

lings were rated for disease reaction based on a 1 to 9 severity scale (3). Disease reactions were recorded as resistant (grades 1 to 3) for those plants with no visible disease symptoms or only a few, very small lesions mostly on primary leaf veins. Plants with numerous enlarged lesions or with sunken cankers on the lower sides of leaves or hypocotyls were recorded as susceptible (grades 4 to 9). Inoculations were repeated at least twice for each isolate. The identified races were assigned a value based on the binary nomenclature system (28). Each differential cultivar had an assigned number (2ⁿ), where *n* corresponded to the place occupied by the cultivar within the differential series. The designation of a race number was obtained by summing the 2ⁿ values of all cultivars exhibiting susceptible reactions to the isolate used for inoculation.

Determination of molecular phenotypes. Single-conidial isolates (60 total) of *C. lindemuthianum* were characterized by RAPD marker data. These included 28 races selected as representative of the full range of virulence phenotypes (Table 1) plus 32 isolates previously reported in Brazil, Canada, Colombia, the Dominican Republic, Guatemala, Mexico, the Netherlands, Peru, and the United States (1,4,6,9,23,30,31,34–36,42) but not found in this survey (Table 2).

DNA extraction of *C. lindemuthianum* races followed the protocol modified from Edwards et al. (11). Mycelium of each race grown in petri dishes containing 20 ml of liquid potato dextrose

medium (20% potato) and an ampicillin solution of 240 µl liter⁻¹ (25 mg ml⁻¹ in 70% EtOH) was used for DNA extraction. Each petri dish was inoculated with four plugs of one single-conidial race that were incubated for 7 days at 22 to 25°C in darkness without shaking. Mycelium was harvested, surface-dried, freeze-dried for 36 h, and stored at –20°C. Dried mycelium was ground to a fine powder with a precooled sterile mortar and pestle. Microcentrifuge tubes (1.5 ml) were filled with 260 mg of ground mycelium that was dispersed in 400 µl of hot (65°C) 2× CTAB extraction buffer (2% CTAB, 100 mM Trizma base, 10 mM EDTA, 0.7 M NaCl). Phenol/chloroform/isoamyl alcohol (400 µl, 24:1, vol/vol) was added, and the mixture was agitated on a shaker for 15 min and centrifuged at 3,000 rpm for 5 min. The aqueous top layer was transferred to a new microcentrifuge tube (1.5 ml) containing 400 µl of isopropanol, mixed well, and incubated at room temperature for 5 min. The emulsion was centrifuged at 3,500 rpm for 5 min, supernatant was discarded, and tubes were inverted for 5 min to allow complete evaporation of the isopropanol. The pellet was dissolved in 100 µl of TE (10 mM Tris-HCl, pH 8.0, 0.5 mM EDTA), RNase A at 10 µg ml⁻¹ (500 µg ml⁻¹) was added, and the emulsion was incubated for 10 min at room temperature. Adding 50 µl of cold 100% EtOH precipitated DNA. After 10 min of incubation at room temperature, the mixture was centrifuged at 3,500 rpm for 5 min, and the pellet was washed twice with EtOH (100%). Extracted DNA was dissolved in 100 µl of TE and stored

TABLE 2. Susceptible (+) and resistant (–) reactions of common bean differential cultivars to races of *Cochliobolus lindemuthianum* previously reported in Brazil, Canada, Colombia, Guatemala, Mexico, the Netherlands, Peru, and the United States characterized by randomly amplified polymorphic DNA

Race ^a	Country ^b	GPC ^c	Differential cultivar ^d												Isolate ^e	Ref. ^f
			A	B ^g	C ^g	D	E	F ^g	G	H	I	J	K	L		
2	Peru	A	–	+	–	–	–	–	–	–	–	–	–	–	CL 52 Per ^h	34
3	Peru, Neth	A	+	+	–	–	–	–	–	–	–	–	–	–	CL 3 Per ^h	34
5*	Peru	A	+	–	+	–	–	–	–	–	–	–	–	–	CL 61 Per ^h	34
7	Peru	A	+	+	+	–	–	–	–	–	–	–	–	–	CL 73 Per ^h	34
15*	Clb	A	+	+	+	+	–	–	–	–	–	–	–	–	CL 262 Clb ^h	34
17	Can, US	M	+	–	–	–	+	–	–	–	–	–	–	–	MSU ⁱ	4,42
19	Neth	A	+	+	–	–	+	–	–	–	–	–	–	–	MSU ⁱ	...
23*	Bra, US, Neth	A	+	+	+	–	+	–	–	–	–	–	–	–	ATCC 18789	1
31*	Bra, Neth	M	+	+	+	+	+	–	–	–	–	–	–	–	3369–M ^j	9,23
38*	DR	A	–	+	+	–	–	+	–	–	–	–	–	–	CL 11 Dom ^h	...
55	Bra	A	+	+	+	–	+	+	–	–	–	–	–	–	CICNF 264 ^k	23
63	...	...	+	+	+	+	+	+	–	–	–	–	–	–	MSU ⁱ	9
64	Mex	M	–	–	–	–	–	–	+	–	–	–	–	–	64 ^l	35
81	Bra, Neth	M	+	–	–	–	+	–	+	–	–	–	–	–	CICNF 538 ^k	23
87*	Bra	M	+	+	+	–	+	–	+	–	–	–	–	–	CICNF 534 ^k	23
102*	Bra, US	A	–	+	+	–	–	+	+	–	–	–	–	–	CICNF 389 ^k	4,6
141	Gua	M	+	–	+	+	–	–	–	+	–	–	–	–	MSU ⁱ	36
130*	Can, US	A	–	+	–	–	–	–	–	+	–	–	–	–	MSU ⁱ	4,42
192*	Mex	M	–	–	–	–	–	–	+	+	–	–	–	–	192 ^l	35
384*	Mex	M	–	–	–	–	–	–	–	+	+	–	–	–	384 ^l	35
453	Bra	M	+	–	+	–	+	–	+	+	–	–	–	–	CICNF 340 ^k	23
1024*	Mex	M	–	–	–	–	–	–	–	–	–	–	+	–	1024 ^l	35
1473*	Mex	M	+	–	–	–	–	–	+	+	+	–	+	–	CI 91 ^h	31
2047*	CR	M	+	+	+	+	+	+	+	+	+	+	+	–	CL 38 CRica ^h	30

^a Designation of races based on the binary nomenclature system (28). Races previously designated by Greek letters: 17, α; 23, δ; 31, κ; 102, γ; 130, β; and 453, ζ.

* indicates isolates analyzed by Fitch-Margoliash cluster analysis (Fig. 1).

^b Bra = Brazil, Can = Canada, Clb = Colombia, CR = Costa Rica, DR = Dominican Republic, Gua = Guatemala, Mex = Mexico, Neth = the Netherlands, and US = the United States.

^c Host gene pool: A = Andean and M = Middle American.

^d Common bean differential cultivars used to characterize races of *C. lindemuthianum* followed by their respective binary value (28): A: Michelite, 1; B: Michigan Dark Red Kidney, 2; C: Perry Marrow, 4; D: Cornell 49242, 8; E: Widusa, 16; F: Kaboon, 32; G: Mexico 222, 64; H: PI 207262, 128; I: TO, 256; J: TU, 512; K: AB 136, 1024; and L: G 2333, 2048. Resistance genes were characterized in cultivars from the common bean differential series (45,47): B: Michigan Dark Red Kidney, *Co-1*; D: Cornell 49242, *Co-2*; G: Mexico 222, *Co-3*; I: TO, *Co-4*; J: TU, *Co-5*; K: AB 136, *Co-6*; and L: G2333, *Co-4*², *Co-5*, and *Co-7*^e.

^e Source (research center and researcher).

^f Reference cited.

^g Cultivars belong to the Andean gene pool.

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^l Mexican National Bean Research Program, INIFAP, Chapingó (R. Rodríguez and J. Acosta).

at -20°C . Concentration of DNA samples was standardized to $10\text{ ng }\mu\text{l}^{-1}$ by DNA fluorometry (TKO 100, Hoefer Scientific Instruments, San Francisco).

The PCR procedure reported by Williams et al. (44) was followed, with minor modifications. Approximately $1.6\text{ }\mu\text{l}$ of genomic DNA template ($10\text{ ng }\mu\text{l}^{-1}$) and $1.6\text{ }\mu\text{l}$ of single-decamer primer ($10\text{ ng }\mu\text{l}^{-1}$) (Operon Technologies Inc., Alameda, CA) was used in a $18.22\text{-}\mu\text{l}$ amplification reaction. The reaction also contained $0.15\text{ }\mu\text{l}$ of Stoffel fragment polymerase ($10\text{ units }\mu\text{l}^{-1}$), $1\times$ Stoffel buffer ($10\times$), 5 mM MgCl_2 (25 mM), and 2 mM dNTPs (200 mM) (Perkin-Elmer Cetus, Norwalk, CT) overlaid with $25\text{ }\mu\text{l}$ of sterile mineral oil prior to amplification. To ensure that amplification products were not primer artifacts (44), genomic DNA was omitted from a control reaction included for each primer examined.

Amplification was performed in a DNA thermal cycler (Perkin-Elmer Cetus 480) programmed for 1 initial denaturation cycle (93°C for 3 min), 40 step-cycles (94°C for 45 s, 38°C for 45 s, and 72°C for 1 min), 1 time-delay cycle (72°C for 10 min), and 1 universal soak cycle (8°C). Amplified RAPD products were electrophoresed at 70 V for 3.5 h on 1.2% agarose gel by $0.5\times$ TBE (45 mM Tris-borate , 1 mM EDTA) running buffer. RAPD products were observed on 0.005% EtBr-stained agarose gel under UV light after electrophoresis. Lambda DNA ($10\text{ }\mu\text{l}$) restricted with *Hind*III and *Eco*RI was included as a molecular weight marker.

A total of 311 primers was initially screened to select polymorphic primers for races 3, 9, 17, 65, 73, 89, 102, 130, 201, 384, 449, and 453 of *C. lindemuthianum*. These races were chosen because of their pathogenicity to the first nine anthracnose differential cultivars. Eight primers were selected (OPA-9, GGGTAACGCC; OPF-13, GGCTGCAGAA; OPG-2, GGCACCTGAGG; OPG-3, GAGCCCTCCA; OPS-17, TGGGGACCAC; OPT-14, AATGCCGCAG; OPV-7, GAAGCCAGCC; and OPV-10, GGACCTGCTG), which consistently generated major polymorphic amplicons in all isolates. Selected primers were tested on all 60 isolates of *C. lindemuthianum*. Molecular polymorphism among isolates with identical virulence phenotypes was investigated. Isolates from races 3, 17, 23, 31, 55, 65, 73, 81, 102, 130, and 453 from the same and different countries and isolates from races 65 and 73 from the same country were tested with eight selected primers. Two replicates of the RAPD assay were run with different template DNA obtained from different DNA isolations.

Data analysis. Virulence and molecular data were analyzed separately by cluster analyses. A single isolate of each unique combination of virulence and molecular phenotypes was included in the analyses.

Separate data matrices were generated for the virulence and molecular data by scoring resistance as 0 and susceptibility as 1 and the absence or presence of a RAPD fragment as 0 or 1, respectively. RAPD amplicons were considered polymorphic if shared by fewer than 57 of the 60 isolates of *C. lindemuthianum*. Similarity matrices for both virulence and molecular data were derived with the Similarity for Qualitative Data Program (SIMQUAL) in the Numerical Taxonomy and Multivariate Analysis System for personal computer (NTSYS-pc) version 1.70 (Exeter Software, Setauket, NY). DICE and Jaccard's coefficients were used to compute distances in virulence and RAPD data, respectively. The ratio between the presence of characters among two individuals and all possible combinations of unmatched characters among the same individuals was considered by both coefficients, although DICE weight matches at twice that of mismatches. The Fitch-Margoliash program in the Phylogeny Inference Package (PHYLIP) version 3.5c (Department of Genetics, University of Washington, Seattle) was used to estimate clustering from the distances matrix data. Phenograms for both virulence and molecular data were produced by the Tree Display Program (Drawgram) in PHYLIP version 3.5c.

RESULTS

Virulence phenotypes. From the 138 *C. lindemuthianum* isolates tested for virulence, 41 races were identified (Table 1). Races fell into two categories: those found over a wide geographic area (i.e., different continents) and those restricted to a single country. Only three races (7, 65, and 73) were widely distributed. Race 73 was the most common, comprising 28% of the total sample. This race was found in four separate countries and was isolated repeatedly in North, South, and Central America. Race 7 was found at low frequencies but was extremely widespread: it was detected once in South and Central America but was isolated repeatedly only from the United States. Race 65 was moderately common (14% of all isolates) but was isolated repeatedly only from Brazil and the United States. The remaining races were detected only in a single country; about one-third of these localized races were isolated repeatedly within a country. These races may be common locally but restricted geographically. Variability was highest in Central America (76%; i.e., number of races detected/number of isolates from the region) and decreased to the north (North America 7%) and south (South America 17%).

Although 39% (16 of 41) of the races were detected repeatedly and 3 races were widespread, no race was isolated from plants from both *P. vulgaris* gene pools. For example, races 65 and 73 were common and widespread, but they were always isolated from hosts of the Middle American gene pool. In contrast, race 7 was isolated from three countries but always from hosts of the Andean gene pool.

Races that were isolated from Middle American hosts were virulent to all cultivars present in the differential series, except cv. L (Table 1). These races were categorized into two groups. One group was virulent to specific resistance genes (*Co-2*, *Co-3*, *Co-4*, *Co-5*, and *Co-6*). The majority of races from this group attacked differentials A and G. The differentials with the *Co-2* and *Co-4* genes mostly were susceptible to races from Honduras and Mexico, respectively. Races 1545, 1600, 1601, 1673, 1929, and 1993 were virulent to both the *Co-5* and *Co-6* genes. Races from Honduras and Mexico were the only races virulent to the *Co-6* gene. Races virulent to genotypes belonging to both gene pools formed the second group. Race 357 was virulent to Andean cvs. C and F and Middle American cvs. G and I. Races 453, 469, 1165, 1431, 1677, and 1741 attacked Andean cv. C. Races 453 and 469 were virulent to Middle American cvs. G, H, and I. Races 1165, 1431, 1677, and 1741 caused disease on Middle American cv. K.

The group of races isolated from Andean hosts was virulent to resistance genes from the Andean and Middle American gene pools. Races 2, 7, and 19 were virulent to the *Co-1* gene in cv. B, whereas race 7 also was virulent to the resistance genes in cv. C. Race 55 was virulent to all three Andean cultivars, B, C, and F. These Andean races also were virulent to Middle American cvs. A and E.

The phenetic analysis included 54 virulence phenotypes of *C. lindemuthianum*: 41 races identified in this survey (Table 1), and 13 additional races previously reported from Brazil, Canada, Colombia, the Dominican Republic, Guatemala, Mexico, the Netherlands, Peru, and the United States (Table 2). The races clustered into three groups, with one group containing a single race, 2047 (Fig. 1). There were no obvious factors that correlated with virulence clusters. The two multiple-isolate clusters contained isolates from hosts of Andean and Middle American ancestry, and both clusters contained isolates from North, South, and Central America. However, isolates from South America were slightly more common in cluster 2. Races that were widespread geographically also were found in clusters 1 and 2.

There were no obvious patterns within clusters 1 and 2, with races obtained from different host gene pools often clustered tightly (e.g., 1033 and 23; 320 and 102; and 31 and 2). No geographic pattern was evident within a cluster, because isolates from each geographic region were spread throughout each cluster.

Molecular variation. Sixty isolates of *C. lindemuthianum* were separated based on amplification product patterns from three hundred twelve combinations of primer-DNA templates. Primers OPA-9, OPF-13, OPG-2, OPG-3, OPS-17, OPT-14, OPV-7, and OPV-10 generated 11, 10, 10, 9, 7, 17, 11, and 18 distinct reproducible polymorphic amplicons, respectively. Ten RAPD amplicons were monomorphic. RAPD data distinguished 15 separate clusters (Fig. 2). The number of isolates within a cluster ranged from 19 for cluster 2 to 1 for 7 separate clusters. There was no congruence between the RAPD and virulence phenograms. Isolates that were identical for virulence (i.e., the same race) were most often dissimilar for RAPD markers. For example, the four isolates that were classified as race 73 were distributed across three clusters within the RAPD phenogram. An example of the RAPD pattern obtained with primer OPA-09 is shown in Figure 3. The analyses of several isolates from races 65 and 73 showed polymorphism within and among countries (Fig. 4). Isolates from

race 73 characterized in Honduras and Mexico showed two distinct RAPD patterns among isolates from each country, whereas only 2 of the 13 isolates from the United States were polymorphic (Fig. 4A). Isolates from race 65 characterized in the United States showed a different RAPD pattern, whereas isolates from Brazil were monomorphic (Fig. 4B). Of the 10 races that displayed intrarace variability for RAPD phenotype, only isolates from races 55 and 453 clustered within the same group of the RAPD phenogram.

The clustering in the RAPD phenogram was not associated with the geography or host gene pool from which the isolate was obtained. The two largest clusters, 1 and 2, contained isolates from all geographic regions sampled that were collected from hosts from both *P. vulgaris* gene pools. Even clusters that contained few isolates were geographically variable. For example, clusters 4, 6, 8, and 13 contained isolates from both Central and South America. Clusters 4 and 8 also contained isolates obtained from hosts of

Fig. 1. Phenogram of *Colletotrichum lindemuthianum* single-spore isolates based on virulence to *Phaseolus* differential cultivars. The DICE coefficient in the Numerical Taxonomy and Multivariate Analysis System for personal computer (NTSYS-pc; Exeter Software) generated distance matrix data of virulence phenotype data. The Fitch-Margoliash program in the Phylogeny Inference Package (PHYILIP) version 3.5c (Department of Genetics, University of Washington) estimated phylogeny from the distance matrix data. **A**, Binary identification (28) of *C. lindemuthianum* races. **B**, Origin of isolates: Arg = Argentina, Bra = Brazil, Clb = Colombia, CR = Costa Rica, DR = the Dominican Republic, Hon = Honduras, Per = Peru, Mex = Mexico, and US = the United States; Div = races identified from isolates collected in different countries. **C**, Middle American (M) and Andean (A) groups of races according to host source of each isolate.

Fig. 2. Phenogram of *Colletotrichum lindemuthianum* single-spore isolates based on molecular polymorphism generated by randomly amplified polymorphic DNA analysis. Jaccard's coefficient in the Numerical Taxonomy and Multivariate Analysis System for personal computer (NTSYS-pc; Exeter Software) generated distance matrix data of virulence phenotype data. The Fitch-Margoliash program in the Phylogeny Inference Package (Phylip) version 3.5c (Department of Genetics, University of Washington) estimated phylogeny from the distance matrix data. **A**, Binary identification (28) of *C. lindemuthianum* races. **B**, Origin of isolates: Bra = Brazil, Can = Canada, Clb = Colombia, CR = Costa Rica, DR = the Dominican Republic, Gua = Guatemala, Hon = Honduras, Per = Peru, Mex = Mexico, and US = the United States. **C**, Middle American (M) and Andean (A) groups of races according to host source of each isolate. * indicates an isolate was obtained from the Michigan State Collection/Bean Breeding and Genetics Laboratory. ** indicates an isolate was obtained from the collection of the Centro Internacional de Agricultura Tropical, Bean Pathology Program.

Andean and Middle American ancestry. The only geographic tendency was for isolates from North America to be found predominantly in clusters 1 and 2.

DISCUSSION

Molecular markers are used extensively to characterize plant pathogens (8,16,24,26). When combined with data on virulence, these markers often can elucidate the population genetic structures and evolutionary relationships of plant pathogens (22,25,39). This information can suggest novel strategies for control of pathogens. For example, rice blast populations, *Pyricularia oryzae*, are often composed of several distinct lineages, with each lineage virulent on a subset of available host cultivars (7,20). Because of this structure, breeding programs have concentrated on obtaining resistance against specific pathogen lineages and not virulence phenotypes (7,20).

The division of common beans, *P. vulgaris*, into Andean and Middle American gene pools (15,38) suggests the possibility of a similar lineage-based control strategy. If pathogens are specialized on one of the host gene pools, then transferring resistance genes between gene pools may provide a more durable resistance (15, 38). Common bean rust (*U. phaseoli*; 21,40,41) and angular leaf spot (*Phaeoisariopsis griseola*; 17) appear to be specialized in congruence with the two *P. vulgaris* gene pools. A previous study of 12 *C. lindemuthianum* isolates found that RAPD and RFLP markers divided the species into two groups (12). Although not absolute, the groupings suggested some specialization within *C. lindemuthianum* that corresponded to the two gene pools within *P.*

vulgaris. Pastor-Corrales (29) reported that *C. lindemuthianum* can be divided into two groups based on virulence: one specializing on hosts from the Middle American gene pool and one specializing on Andean gene pool hosts.

Our data for virulence phenotypes and RAPD markers suggest that *C. lindemuthianum* is not structured in congruence with host gene pools. Virulence phenotypes clustered predominantly into two large groups, but each group contained races isolated from both of the host gene pools. The virulence data also can be examined for the ability of races isolated from hosts from one gene pool to infect differential cultivars from the other gene pool. Differential cvs. B, C and F, which represent the Andean gene pool, were susceptible to 3, 17, and 3%, respectively, of the 37 races isolated from hosts of Middle American origin (Table 1). Further, some of the isolates previously collected from Middle American hosts infected these Andean cultivars (Table 2). Three of the four *C. lindemuthianum* races obtained from Andean hosts could infect at least one differential cultivar from the Middle American gene pool (Table 1). These results, however, are based on greenhouse inoculations. Despite the ability of some races to overcome resistance genes from both the Middle American and Andean host gene pools, each race was actually collected from hosts of only one gene pool.

A previous study reported that widespread races (i.e., races found in five or more Latin American countries) of *C. lindemuthianum* infected a smaller number of differential cultivars (29). Our data tend to agree with this finding; the three widespread races, 7, 65, and 73, could attack two or three differentials, whereas the average number of differentials infected by a race was 3.73. Although our sampling was not designed to explicitly measure diversity within an area, our data suggest that *C. lindemuthianum* is more variable in Central America than in either South or North America. This finding is consistent with the earlier work of Pastor-Corrales (29) who found that the *C. lindemuthianum* population in Central America was more diverse than isolates from Andean areas. Also, genetic diversity of *C. lindemuthianum* populations was higher in Mexico compared to Ecuador and Argentina (37).

RAPD markers separated our isolates into 15 clusters. The clustering within the RAPD phenogram was not congruent with either geographic location or host gene pool nor was there any congruence with the virulence phenogram. Thus, the RAPD primers utilized here cannot be used for grouping *C. lindemuthianum* according to the host gene pool. It is not clear to what degree our RAPD phenogram represents phylogenetic relationships within *C. lindemuthianum*. Recent reports have pointed out a number of problems associated with assessing phylogenetic relationships based on RAPD markers (27). Our groupings differ from an earlier report based on 12 *C. lindemuthianum* isolates (12), which found 2 groups using both RAPD markers and two sets of RFLP markers from the ribosomal genes. Sicard et al. (37) grouped *C. lindemuthianum* iso-

Fig. 3. Randomly amplified polymorphic DNA amplicons obtained with primer 9 of Operon kit A for 17 single-spore isolates of *Colletotrichum lindemuthianum*: lane a, 17 (α , US [the United States]); lane b, 17 (α , Can [Canada]); lane c, 19 (ϵ -Kenya, Neth [the Netherlands]); lane d, 89 (α -Brazil, Bra [Brazil]); lane e, 89 (α -Brazil, Neth); lane f, 130 (β , US); lane g, 130 (β , Can); lane h, 130 (β , Neth); lane i, 23 (δ , US); lane j, 23 (δ , Neth); lane k, 102 (γ , US); lane l, 31 (κ , Neth); lane m, 31 (κ , Bra); lane n, 55 (λ , the Dominican Republic); lane o, 63 (ι , CIAT); and lane p, 453 (ζ , Mexico). Races were grouped according to Andean (A) and Middle American (M) reactions.

Fig. 4. Randomly amplified polymorphic DNA patterns obtained with primer 13 of Operon kit F for 19 single-spore isolates from **A**, races 73 and **B**, 65 of *Colletotrichum lindemuthianum* collected from different geographic regions. **A**, Lanes a through d, Honduras; lanes e and f, Mexico; and lanes g through r, United States. **B**, Lanes a through f, United States; and lanes g through k, Brazil.

lates by RFLP of the amplified ribosomal internal transcribed spacer region. The authors (37) demonstrated that the three groups corresponded to the host gene pools. These results suggest an adaptation of strains on cultivars of the same geographic origin.

Taken together, our data indicate that *C. lindemuthianum* may not be highly structured to specific *P. vulgaris* gene pools. Therefore, control strategies that transfer resistance genes from one *P. vulgaris* gene pool to the other may not confer durable resistance to *C. lindemuthianum*. We urge caution in the utilization of this strategy until races are further evaluated under field conditions for their ability to infect hosts from both gene pools and a more comprehensive analysis of the evolutionary relationships within *C. lindemuthianum* is completed.

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