Characterization of Variability in the Fungus *Phaeoisariopsis griseola* Suggests Coevolution with the Common Bean (*Phaseolus vulgaris*)

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This research was supported by a grant from the USAID Bean/Cowpea Cooperative Research Support Program (CRSP) to the second, fifth, seventh, and eighth authors; and by a fellowship from the Organization of American States (OAS) to the first author.

Reprint requests to the second author.

We thank A. Sartorato, L. Zambolim, J. A. Ventura, and C. Kurowski for angular leafspot samples, and J. Hall for assistance in preparation of the figures.

Accepted for publication 6 February 1995.

ABSTRACT

Guzmán, P., Gilbertson, R. L., Nodari, R., Johnson, W. C., Temple, S. R., Mandala, D., Mkandawire, A. B. C., and Gepts, P. 1995. Characterization of variability in the fungus *Phaeoisariopsis griseola* suggests coevolution with the common bean (*Phaseolus vulgaris*). Phytopathology 85:600-607.

Angular leaf spot (ALS) disease of common bean (*Phaseolus vulgaris*) is caused by the imperfect fungus *Phaeoisariopsis griseola* and severely reduces bean yields in tropical and subtropical countries. Breeding for disease resistance has been difficult because there is substantial pathogenic variation among fungal isolates. Random amplified polymorphic DNA (RAPD) markers were used to characterize 62 *P. griseola* isolates from three countries (Malawi, the United States, and Brazil). The gene pool of the bean plants from which the isolates were obtained was determined by isozyme and phaseolin analysis. Eleven primers generated reproducible and distinct RAPD patterns that divided the *P. griseola* isolates into two major groups. Group 1 (Andean) isolates were generally recovered from Andean gene pool materials, whereas group 2 (Mesoamerican) isolates were recovered from Mesoamerican materials. *Phaeoisariopsis griseola* isolates representing groups 1 and 2 were inoculated onto selected Andean and Mesoamerican bean genotypes. Group 1 isolates were more pathogenic on Andean beans, whereas group 2 isolates were more pathogenic on Mesoamerican beans. RAPD and pathogenicity data suggest that groups 1 and 2 may have originated in the Andes and Mesoamerica, respectively, and that coevolution of the *P. griseola* fungus and its common bean host has resulted in increased levels of disease in this host-pathogen interaction. The results have implications in development of ALS breeding strategies.

Additional keyword: host-pathogen coevolution.

The interaction between host plants and pathogens is controlled by genes in both organisms (10). Whether the interaction between host and pathogen is compatible or not will affect the probability of survival of the host or the pathogen and therefore will alter gene frequencies, particularly of those genes involved in the interaction. Therefore, coevolution between a plant host and a pathogen can be interpreted as a correlated change in their gene frequencies. It has been generally assumed that this change occurs in the direction of lower levels of disease severity (11). Alternatively, evolution towards a higher degree of specialization between host and pathogen has been posited (33). In order to obtain additional information on the phenomenon of coevolution between host and pathogen, we have investigated the relationship between genetic variation in the common bean (*Phaseolus vulgaris* L.) and that in the causal agent of angular leafspot disease (ALS), the fungus *Phaeoisariopsis griseola* (Sacc.) Ferraris.

Phaeoisariopsis griseola causes ALS on common bean in more than 60 countries worldwide, and yield losses may reach 80% (5,7,8,36,37). Thus, breeding for disease resistance to ALS is a priority of the national bean breeding programs in several East African countries, as well as in some Central and South American countries. Preliminary evidence for variability among *P. griseola* isolates was reported in the early 1950s (2). More conclusive evidence of pathogenic variability has been shown based on differential pathogenicity in bean cultivars (1). In a recent study using isozyme analysis, 55 *P. griseola* isolates from the United States, Latin America, and Africa were divided into two groups (6). All African isolates displayed pattern 1 for each isozyme, whereas Latin American isolates exhibited either pattern 1 or 2. Pattern 1 isolates were associated with large-seeded bean cultivars originating in the Andean region of South America, whereas pattern 2 isolates were associated with small-seeded bean cultivars from Central and North America (6).

Based on several lines of evidence, including seed protein analysis, allozyme marker studies, restriction fragment length polymorphism and random amplified polymorphic DNA (RAPD) analysis, morphological and agronomic characters, and incidence of genes for hybrid lethality, cultivated common beans have been separated into two distinct groups called the Andean and Mesoamerican gene pools (12,13,14,38,39). These gene pools appear to have arisen, one in the Andes mountains of South America and the other in southern or western Mexico and/or Central America, from independent domestication events (16,17). Wild populations of *P. vulgaris* can also be separated into these two major gene pools, and the geographic pattern of morphological and biochemical diversity and of reproductive isolation barriers found in cultivated beans parallels that of their wild relatives (23,24). This suggests that the divergence of the two gene pools occurred prior to independent domestication events (16). Gepts and Bliss (14) hypothesized that the existence of two host gene pools might be paralleled by the existence of two gene pools in organisms associated with common bean, such as pathogens or symbionts.

RAPD markers have been used extensively during the last several years to analyze genetic diversity of plants (9,21,42,43) and plant pathogens (19,20), to identify polymorphisms tightly linked to disease-resistance genes (29,30,32), and for genome mapping (34). In this study, we have characterized the genetic diversity

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among *P. griseola* isolates from different bean growing regions at the molecular (RAPD) and phenotypic (host-pathogen interaction) levels. Our results show that these isolates can be organized into two groups, one comprising isolates from Mesoamerican bean genotypes and the other comprising isolates from Andean bean genotypes, and support the hypothesis of coevolution between common bean and *P. griseola*. By providing a way to identify the evolutionary origin of pathogen isolates, breeders can more readily identify host plant genotypes with ALS resistance.

MATERIALS AND METHODS

Fungal isolates. A total of 62 *P. griseola* isolates were recovered from leaf samples showing symptoms of ALS: 44 from the northern, central and southern bean-growing regions of Malawi, Africa; seven from Wisconsin in the United States; and 11 from two bean-growing regions of Brazil (Table 1). Individual leaves with ALS lesions were placed on sterile glass rods in petri plates lined with moistened paper towels. Leaves were incubated for 2 days at room temperature to induce fungal sporulation. Conidia from sporulating lesions were directly transferred with a dissecting needle to V8-juice agar and cultures grown from individual conidia at 25°C. Each *P. griseola* isolate was recovered from a different infected bean plant.

DNA isolation. Erlenmeyer flasks containing V8 liquid medium (100 ml) were inoculated with three agar plugs from 10-day-old sporulating cultures of each *P. griseola* isolate. Flasks were covered with aluminum foil and placed on a shaker (150 rpm) at room temperature for at least 12 days. Mycelium was harvested by filtering cultures through Miracloth (Calbiochem, La Jolla, Calif.), and lyophilized for 24 h in a Virtis lyophilizer (Virtis, Gardiner, N.Y.).

Total genomic DNA was extracted from *P. griseola* mycelium by a phenol/chloroform extraction procedure. Mycelium (0.1 g) was ground in liquid nitrogen into a fine powder with a mortar and pestle. Two milliliters of extraction buffer (0.5 M NaCl, 10 mM Tris-HCl pH 7.5, 10 mM EDTA, 1% sodium dodecyl sulfate [SDS]) were added and mixed to form a slurry. Three milliliters of phenol/chloroform (1:1) were added and the suspension was thoroughly mixed. This suspension was centrifuged (12,000 × *g*) for 20 min at 4°C. The supernatant was reextracted with 3 ml of chloroform, and centrifuged (12,000 × *g*) for 10 min at 4°C. The supernatant was collected, and nucleic acids were precipitated by addition of 0.5 volume of isopropanol and kept overnight at –20°C. Nucleic acids were recovered by centrifugation (12,000 × *g*) for 15 min at 4°C. The nucleic acid pellet was washed with 5 ml of 70% ethanol (12,000 × *g*, 4°C, for 5 min) and air-dried or dried in a speed-vacuum dryer (Savant, Farmingdale, N.Y.). The nucleic acids were resuspended in Tris-EDTA (TE) buffer (10 mM Tris-HCl pH 7.5, 1 mM EDTA). Total genomic DNA was quantified using a TKO 100 Fluorometer (Hoefer, San Francisco, Calif.), and adjusted to a final concentration of 10 ng per ml for RAPD analysis.

RAPD analysis and PCR. Twenty 10-mer primers from Kit A of Operon Technologies (Alameda, Calif.) were tested for their ability to reveal polymorphisms among *P. griseola* isolates. The total volume of the polymerase chain reaction (PCR) reaction was 50 µl. Unless stated otherwise, all values for concentrations of the chemicals correspond to the final concentrations. Reactions were prepared by mixing 26.5 µl of distilled water, 5 µl buffer (10 mM Tris-HCl, 50 mM KCl), 3 µl $MgCl_2$ (2.5 mM), 2 µl Gelatin (0.01%), 2 µl Triton X-100 (0.1%), 4 µl mixed dNTPs (0.2 mM each), 0.5 µl Taq polymerase (2.5 U), 2 µl primer (1.0 mM), and 5 µl total genomic DNA (10 ng per ml). Amplification was performed in a DNA Thermal Cycler 480 (Perkin-Elmer Cetus, Emeryville, Calif.). The amplification profile was 45 cycles of 45 s at 92 C, 60 s at 34 C, and 90 s at 72 C. The last cycle was followed by a final extension step of 10 min at 72 C. Fifteen-microliter samples of the RAPD products were examined by electrophoresis in 1.5% agarose gels in 0.5X Tris-borate EDTA (TBE) buffer at 96 V for 3 h, stained with ethidium bromide, and visualized on an ultraviolet transilluminator (Fisher Scientific, Pittsburgh, Pa.). All analyses included a negative control in which no template DNA was included in the amplification reaction mixture.

Phenetic analysis of RAPD results. RAPD bands were scored as present (1) or absent (0) to generate a data matrix. This matrix was then analyzed by the SIMQUAL program of NTSYS-pc, version 1.60 (35), to generate a simple matching coefficient (m/n), where m = number of matches and n = total number of bands. An unweighted pair group arithmetic mean method (UPGMA) cluster analysis was performed based on the matching coefficient using the SAHN program of NTSYS. The UPGMA clustering method was chosen because it is the most widely used method and provides results most consistent with pedigree information (31). Finally, a dendrogram was generated using the TREE program of NTSYS.

Bean gene pool characterization. The gene pool of the bean plants was determined by allozyme and phaseolin seed protein analysis. Allozyme analyses were conducted on a crude homogenate of primary leaf or root tissue from 10-day-old seedlings as described (22). Five enzyme systems were used: aconitase (ACO), diaphorase (DIAP), malic enzyme (ME), ribulose bisphosphate carboxylase (small subunit, RBCS), and shikimate dehydrogenase (SKDH). Allozyme loci and alleles were designated as described (22). Phaseolin seed protein was analyzed using the SDS–polyacrylamide gel electrophoresis (PAGE) system of Laemmli (25) as modified by Ma and Bliss (28).

Inoculation of beans with *P. griseola*. Four *P. griseola* isolates from Malawi (PgMal 4, PgMal 7, PgMal 30, and PgMal 38), and one isolate from Brazil (PgBr 8) were inoculated onto 24 bean genotypes (representing Andean and Mesoamerican gene pool materials) from Malawi under greenhouse conditions. In addition, these isolates were inoculated onto two resistant lines (A 240 and A 286) obtained from the Centro Internacional de Agricultura Tropical (CIAT) and two susceptible commercial snap bean cultivars (cvs. Topcrop and Sutter Pink).

Inoculum was produced by inoculating petri dishes containing V8-juice agar with a concentrated suspension of conidia prepared from a 10-day-old culture of each isolate. A multipoint inoculation technique was used to seed petri dishes. Inoculum was uniformly distributed over the plate by rotating the plates by hand. Plates were incubated for 10 days in the dark at 25°C. Inoculum was prepared from plates by adding sterile distilled water and scraping the surface of the plate with a spatula. Spore concentrations were determined with a hemacytometer and adjusted to 2×10^4 conidia per ml.

Inoculum was sprayed onto the upper and lower leaf surfaces of fully expanded first trifoliate leaves (V3 stage) with a hand atomizer attached to a Spra-tool (Crown Industrial Products, Hebron, Ill.). Plants were covered with plastic bags for 6 days after inoculation and kept in a greenhouse at 25 to 28°C. Bags were removed and plants kept in the greenhouse until ALS disease severity was evaluated 12 days after inoculation on a 1 to 9 rating scale with 1 representing immunity and 9 representing extreme susceptibility (4). The inoculation experiment was repeated three times.

RESULTS

RAPD analysis of *P. griseola* isolates. Initially, RAPD analysis was carried out with six Operon Kit A (OA) primers (OA1, OA4, OA8, OA13, OA18, OA20) on total genomic DNA extracted from 24 *P. griseola* isolates: one isolate from Wisconsin (U.S.) (PgWi 1, isolated from an Andean [red kidney] gene pool bean), one isolate from Goiania (Brazil) (PgBr 1, isolated from a presumed Mesoamerican gene pool bean), and 22 isolates from

TABLE 1. Origin and gene pool classification of the common bean (*Phaseolus vulgaris*) plants and origin and random amplified polymorphic DNA (RAPD) group of corresponding *Phaeoisariopsis griseola* isolates recovered from these plants

				Isozyme,[c] phaseolin,[d] and gene pool[e] classification (*P. vulgaris*)								RAPD group
P. vulgaris[a]	*P. griseola*[a]	Collection site	Collector[b]	*Aco-2*	*Diap-1*	*Diap-2*	*Me*	*Rbcs*	*Skdh*	*Phs*	Gene pool	(*P. griseola*)[f]
Mal1	PgMal 1	Ntchisi	DM	–[g]	100	–	98	98	100	T	A	1
Mal2	PgMal 2	Thyolo	DM	–	100	–	98	98	100	T/C	A	1
Mal3	PgMal 3	Dedza	DM	–	100	–	98	98	100	T	A	1
Mal4	PgMal 4	Dedza	DM	–	**100**[h]	–	102	100	103	S	H[h]	2
Mal7	PgMal 7	Mzimba	DM	–	100	–	102	100	103	T	A	2
Mal18	PgMal 18	Mangochi	DM	–	100	–	98	98	100	C	A	1
Mal19	PgMal 19	Mangochi	DM	–	100	–	98	98	100	C	A	1
Mal20	PgMal 20	Mangochi	DM	–	100	–	98	98	100	T	A	1
Mal22	PgMal 22	Dowa	DM	–	100	–	98	98	100	T	A	1
Mal23	PgMal 23	Dowa	DM	–	**95**	–	**102**	98	100	C	H	1
Mal24	PgMal 24	Dowa	DM	–	100	–	98	98	100	T	A	1
Mal25	PgMal 25	Chitipa	RLG	–	–	–	–	–	–	–	–	1
Mal26	PgMal 26	Rumphi	RLG	–	100	–	98	98	100	T	A	1
Mal28	PgMal 28	Rumphi	RLG	–	100	–	98	98	100	T	A	1
Mal29	PgMal 29	Rumphi	RLG	–	100	–	–	98	100	T	A	1
Mal30	PgMal 30	Rumphi	RLG	–	95	–	–	98	103	S	M	1
Mal33	PgMal 33	Rumphi	RLG	–	100	–	–	98	100	T	A	1
Mal34	PgMal 34	Rumphi	RLG	–	–	–	–	–	–	T	–	1
Mal35	PgMal 35	Rumphi	RLG	–	95	–	102	100	103	S	M	1
Mal36	PgMal 36	Lilongwe	RLG	–	100	–	98	98	100	T	A	1
Mal38	PgMal 38	Chitipa	RLG	–	100	–	98	–	**103**	T	H	1
Mal39	PgMal 39	Chitipa	RLG	–	100	–	98	**100**	100	T	H	1
Mal40	PgMal 40	Mzimba	ST	–	–	–	–	–	–	–	–	1
Mal41	PgMal 41	Mzimba	ST	–	–	–	102	–	103	S	M	1
Mal42	PgMal 42	Dedza	ST	–	–	–	98	–	100	T	A	1
Mal45	PgMal 45	Mzimba	ST	–	95	–	102	–	103	S	M	1
Mal46	PgMal 46	Ntchisi	ST	–	95	–	102	–	103	S	M	2
Mal48	PgMal 48	Mzimba	ST	–	100	–	98	–	100	T	A	1
Mal49	PgMal 49	Lilongwe	ST	–	–	–	–	–	–	–	–	1
Mal50	PgMal 50	Mzimba	ST	–	**100**	–	102	–	103	S	H	1
Mal51	PgMal 51	Mzimba	ST	–	95	–	100	–	103	S	M	1
Mal52	PgMal 52	Mzimba	ST	–	100	–	98	–	100	T	A	1
Mal53	PgMal 53	Mzimba	ST	–	95	–	100	–	–	S	M	1
Mal55	PgMal 55	Ntchisi	ST	–	100	–	98	–	100	T	A	1
Mal56	PgMal 56	Ntchisi	ST	–	100	–	98	–	100	T	A	1
Mal59	PgMal 59	Ntchisi	ST	–	100	–	–	–	100	T	A	1
Mal60	PgMal 60	Ntchisi	ST	–	100	–	98	–	100	T	A	1
Mal61	PgMal 61	Ntchisi	ST	–	100	–	98	–	100	T	A	1
Mal63	PgMal 63	Mzimba	ST	–	100	–	100	–	–	T	A	1
Mal64	PgMal 64	Mzimba	ST	–	100	–	–	–	–	T	A	1
Mal69	PgMal 69	Ntchisi	ST	–	100	–	98	–	–	T	A	1
Mal72	PgMal 72	Dedza	ST	–	100	–	100	–	103	S	A	1
Mal80	PgMal 80	Dedza	ST	–	100	–	98	–	100	T	A	1
Mal81	PgMal 81	Dedza	ST	–	100	–	98	–	100	T	A	1
RK	PgWi 1		RLG	–	–	–	–	–	–	–	A	1
RK	PgWi 2		CK	–	–	–	–	–	–	–	A	1
RK	PgWi 3		CK	–	–	–	–	–	–	–	A	1
RK	PgWi 4		CK	–	–	–	–	–	–	–	A	1
RK	PgWi 5		CK	–	–	–	–	–	–	–	A	1
RK	PgWi 6		CK	–	–	–	–	–	–	–	A	1
RK	PgWi 7		CK	–	–	–	–	–	–	–	A	1
Unknown	PgBr 1	Goiania	AS	100	95	105	–	100	100	S	M	2
Rio Tibagi	PgBr 4	SP Arace	LZ	100	95	100	–	100	100	S	M	2
Serrano	PgBr 5	SP Arace	LZ	100	95	100	–	100	100	S	M	2
0155	PgBr 6	AF.Claudio	JV	100	95	100	–	100	100	S	M	2
Serrano	PgBr 7	AF.Claudio	JV	100	95	100	–	100	100	S	M	2
Rio Tibagi	PgBr 8	AF.Claudio	JV	100	95	100	–	100	100	S	M	2
Serrano	PgBr 10	Ven. Nova	JV	100	–	–	–	–	–	S	M	2
AN910902	PgBr 11	SP Arace	LZ	100	95	105	–	100	100	S	M	2
0155	PgBr 12	SP Arace	LZ	100	95	100	–	100	100	S	M	2
Capixaba precoce	PgBr 13	SP Arace	LZ	100	95	105	–	100	100	S	M	2
0155	PgBr 14	Ven. Nova	JV	100	95	100	–	100	100	S	M	2

[a] Pg = *P. griseola*; Br = Brazil; Mal = Malawi; Wi = Wisconsin; RK = red kidney.

[b] AS: Aloisio Sartorato; CK: Chester Kurowski; DM: Dexter Mandala; JV: Jose Aires Ventura; LZ: Laercio Zambolim; RLG: Robert L. Gilbertson; ST: Steven Temple.

[c] Isozyme loci and alleles as defined by Koenig and Gepts (23) and Singh et al. (38).

[d] Phaseolin types as defined in Gepts et al. (18).

[e] Host gene pool: A= Andean; M= Mesoamerican.

[f] *P. griseola* groups as defined in text: 1= Andean; 2= Mesoamerican.

[g] – : not determined.

[h] Marker alleles in bold suggest hybridity (H) between the two gene pools.

Malawi, isolated from either Andean or Mesoamerican gene pool beans (PgMal 1, PgMal 2, PgMal 3, PgMal 4, PgMal 7, PgMal 18, PgMal 19, PgMal 20, PgMal 22, PgMal 23, PgMal 24, PgMal 25, PgMal 26, PgMal 28, PgMal 29, PgMal 30, PgMal 33, PgMal 34, PgMal 35, PgMal 36, PgMal 38, and PgMal 39). All six primers amplified from 2 to 8 distinct DNA bands from all 24 isolates, indicating that the extraction procedure yielded total genomic DNA suitable for RAPD analysis. Furthermore, based on RAPD patterns generated with these primers, the isolates were separated into two major groups: group 1 (PgWi 1, PgMal 1, PgMal 2, PgMal 3, PgMal 18, PgMal 19, PgMal 20, PgMal 22, PgMal 23, PgMal 24, PgMal 25, PgMal 26, PgMal 28, PgMal 29, PgMal 30, PgMal 33, PgMal 34, PgMal 35, PgMal 36, PgMal 38, and PgMal 39) and group 2 (PgBr 1, PgMal 4 and PgMal 7). Nine of the 14 additional OA primers (OA2, OA3, OA5, OA7, OA9, OA10, OA11, OA14, OA15) consistently generated from 2 to 8 distinct DNA bands and separated the isolates into the two previously identified groups (Fig. 1). Of the remaining five primers, four (OA12, OA16, OA17, and OA19) did not give consistent results among different amplification reactions, and one (OA6) did not reveal any polymorphism among the isolates.

Based on these results, 11 primers (OA1, OA2, OA3, OA4, OA5, OA7, OA9, OA10, OA11, OA14, OA15), which generated the most distinct and reproducible polymorphisms, were selected to characterize all 62 *P. griseola* isolates. All of these primers separated the 44 isolates of *P. griseola* from Malawi into the two groups, with 41 isolates belonging to group 1 and three isolates belonging to group 2 (Fig. 2). All 11 primers identified the 11 isolates from Brazil as group 2 isolates (Fig. 3), whereas all of the Wisconsin isolates were identified as group 1 isolates (data not shown). Analysis of the data matrix generated based on the RAPD bands confirmed the grouping of these isolates into two major groups (Figs. 4 and 5).

Bean gene pool analysis and correlation with *P. griseola* RAPD groups. The bean gene pool of the plants from which all 62 *P. griseola* isolates were collected was determined based on isozyme and phaseolin seed protein phenotype (Table 1). In a few cases, the gene pool of the host plants was inferred based on previous knowledge. For example, it was inferred that PgBr 1 was isolated from Mesoamerican material because most of the beans grown in Brazil are of Mesoamerican origin (18); and red kidney beans, from which all the Wisconsin isolates were recovered, are a known Andean material (3,18).

The two *P. griseola* groups were correlated with the host gene pool (Table 1). Of the 44 group 1 isolates for which the bean gene pool was determined or inferred, 34 were recovered from Andean

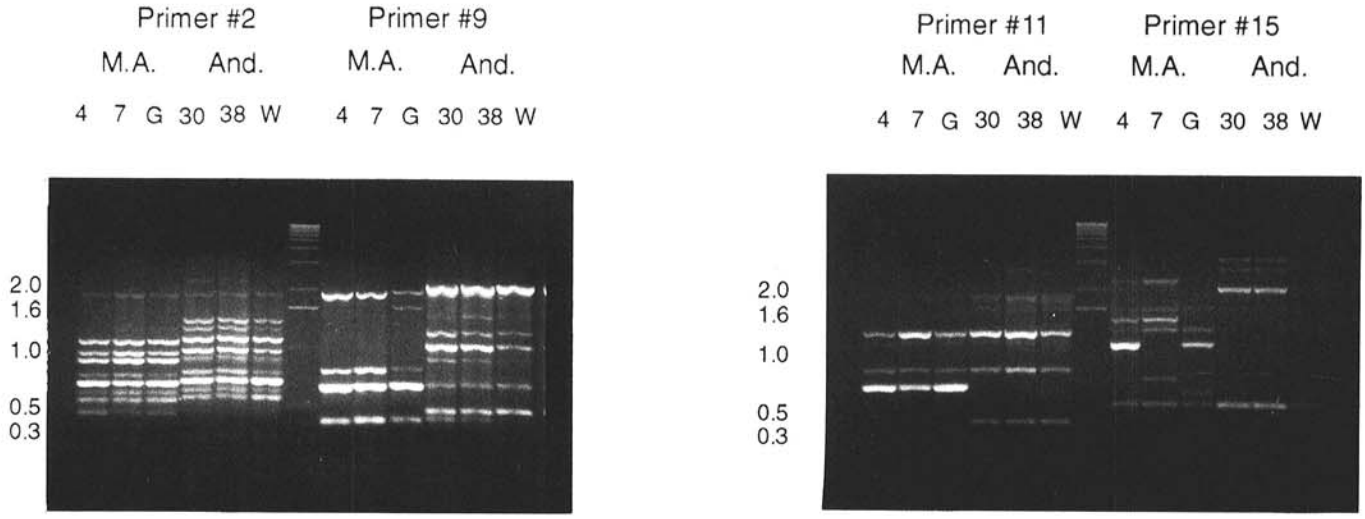

Fig. 1. Random amplified polymorphic DNA (RAPD) patterns obtained with primers 2, 9, 11, and 15 of Operon Kit A for six isolates of *Phaeoisariopsis griseola* including group 1 (Andean, And.) isolates PgMal 30 (30), PgMal 38 (38), and PgWi 1 (W), and group 2 (Mesoamerican, M.A.) isolates PgMal 4 (4), PgMal 7 (7) and PgBr 1 (G). Numbers on the left side indicate the fragment sizes of the 1 kb ladder (GIBCO BRL, Gaithersburg, Md.) in lane 7.

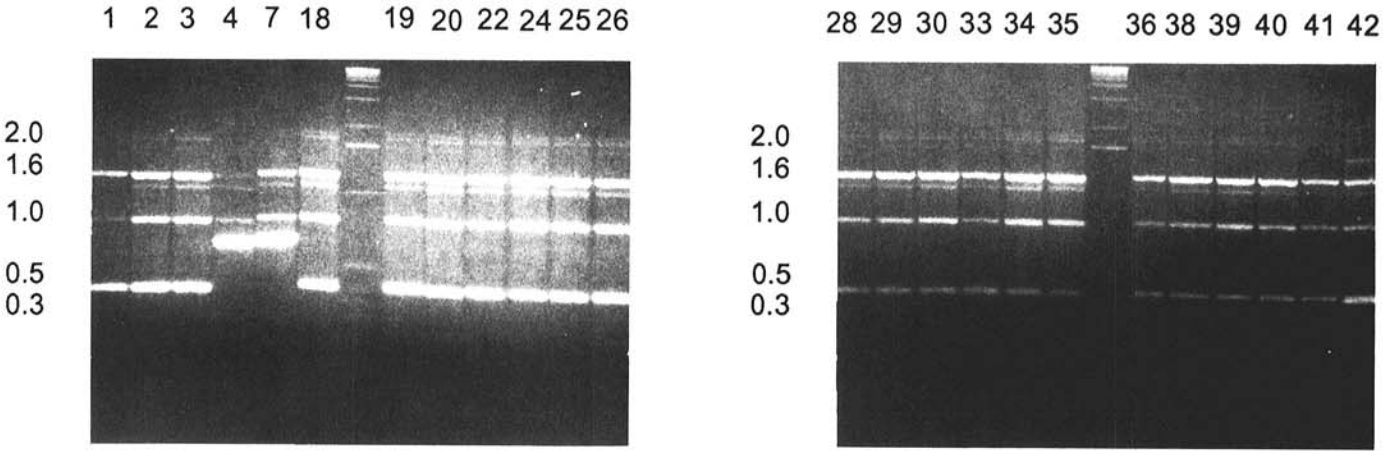

Fig. 2. Random amplified polymorphic DNA (RAPD) patterns obtained with primer 11 of Operon Kit A for 24 isolates of *Phaeoisariopsis griseola* from Malawi. Numbers at the top indicate the PgMal isolate designations. Isolates 1, 2, 3, 18, 19, 20, 22, 24, 25, 26, 28, 29, 30, 33, 34, 35, 36, 38, 39, 40, 41, and 42 represent group 1 (Andean) isolates, whereas isolates 4 and 7 represent group 2 (Mesoamerican) isolates. Numbers on left side indicate the fragment sizes of the 1 kb ladder (GIBCO BRL, Gaithersburg, Md.) in lane 7.

gene pool beans, six were from Mesoamerican gene pool beans, and four were from beans that are supposed hybrids between the two host gene pools based on phaseolin and isozyme data (Table 1). Of the 14 group 2 isolates, 12 were recovered from Mesoamerican gene pool beans, one from an Andean gene pool bean, and one from a hybrid.

Furthermore, group 1 and group 2 *P. griseola* isolates predominated in different geographical regions according to the predominant bean gene pool material in these regions. In Malawi, 28 of the 40 bean genotypes analyzed for isozyme and phaseolin variation were determined to be Andean gene pool materials (Table 1). The other genotypes were either of Mesoamerican origin or hybrids between the Mesoamerican and Andean gene pools. Forty-one of the 44 *P. griseola* isolates from Malawi were group 1 isolates. Similarly, all seven of the Wisconsin isolates, which were from Andean gene pool beans, also were group 1 isolates. In contrast, all the bean genotypes from Brazil were Mesoamerican gene pool materials (Table 1), and all the *P. griseola* isolates recovered from these materials were group 2 isolates. Based on these results, group 1 *P. griseola* isolates can be tentatively referred to as Andean isolates, whereas group 2 isolates can be referred to as Mesoamerican isolates.

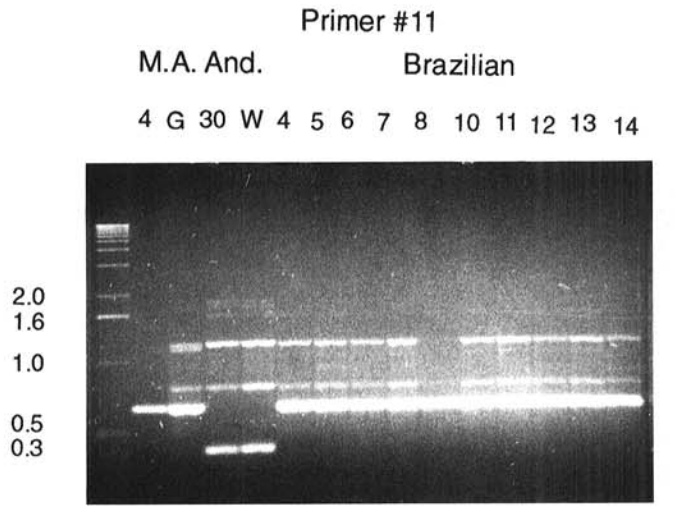

Fig. 3. Random amplified polymorphic DNA (RAPD) patterns obtained with primer 11 of Operon Kit A for 11 isolates of *Phaeoisariopsis griseola* from Brazil, group 1 (Andean, And.) isolates PgMal 30 (30) and PgWi 1 (W), and group 2 (Mesoamerican, M.A.) isolates PgMal 4 (4) and PgBr 1 (G). Numbers on top indicate the isolate designations, and numbers on the left side indicate the fragment sizes of the 1 kb ladder (GIBCO BRL, Gaithersburg, Md.) in lane 1.

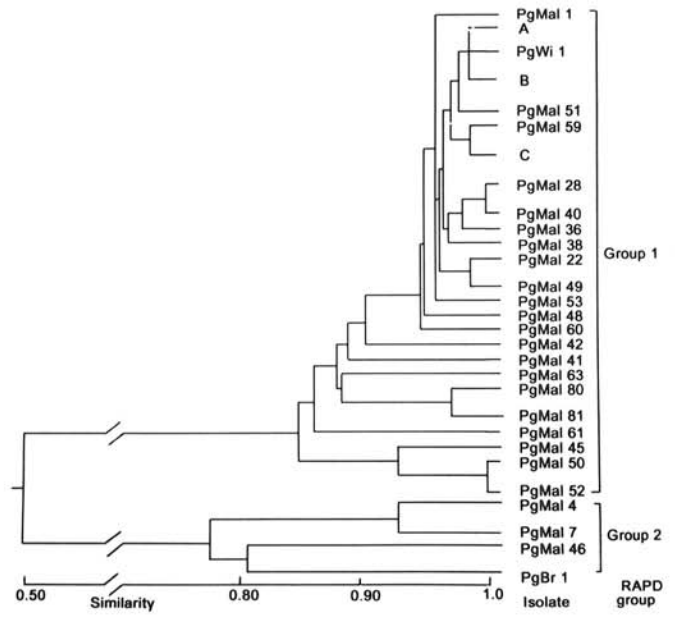

Fig. 4. Dendrogram generated based on random amplified polymorphic DNA (RAPD) fragments from 42 isolates of *Phaeoisariopsis griseola* from Malawi, a representative group 1 (Andean) isolate from the United States (PgWi 1), and a representative group 2 (Mesoamerican) isolate from Brazil (PgBr 1). "A" represents 14 isolates with identical RAPD patterns (PgMal 2, PgMal 3, PgMal 18, PgMal 20, PgMal 23, PgMal 24, PgMal 25, PgMal 26, PgMal 29, PgMal 30, PgMal 33, PgMal 34, PgMal 55, and PgMal 56); "B" represents three isolates with identical RAPD patterns (PgMal 19, PgMal 35, and PgMal 39), and "C" represents three isolates with identical RAPD patterns (PgMal 64, PgMal 69, and PgMal 72).

Host-pathogen interaction. The correlation in genetic diversity patterns between *P. griseola* groups (as determined by RAPD analysis) and the common bean host gene pools raised the issue of whether these associations were correlated with the degree of compatibility of the host-pathogen interaction. Five randomly chosen *P. griseola* isolates representing the two *P. griseola* groups (group 1 [Andean] isolates PgMal 30 and PgMal 38; and group 2 [Mesoamerican] isolates PgMal 4, PgMal 7, and PgBr 8) were inoculated onto 24 bean genotypes from Malawi, representing Andean and Mesoamerican materials (Table 2).

The pathogenicity tests were standardized by including four cultivars/lines, two of which are known to be highly susceptible to *P. griseola* (cvs. Topcrop and Sutter Pink) and two that are known to be resistant (lines A 240 and A 286). These cultivars/lines gave the expected reaction upon inoculation with each of the five isolates (Table 2), indicating the pathogenicity of these isolates and the suitability of the test to reveal resistance. The group 1 (Andean) isolates, PgMal 30 and PgMal 38, were generally more pathogenic on Andean genotypes and less pathogenic on Mesoamerican genotypes (Table 2; Fig. 6). Conversely, the group 2 (Mesoamerican) isolates, PgMal 4 and PgMal 7, were more pathogenic on Mesoamerican genotypes and less pathogenic on Andean genotypes. The group 2 (Mesoamerican) isolate PgBr 8, from Brazil, also was more pathogenic on Mesoamerican than Andean genotypes (Table 2). Overall, an average disease rating of 7 was obtained when group 1 (Andean) isolates were inoculated onto Andean genotypes, versus an average disease rating of 4 when these isolates were inoculated onto Mesoamerican genotypes. Conversely, an average disease rating of 8 was obtained when group 2 (Mesoamerican) isolates were inoculated onto Mesoamerican genotypes, versus an average disease rating

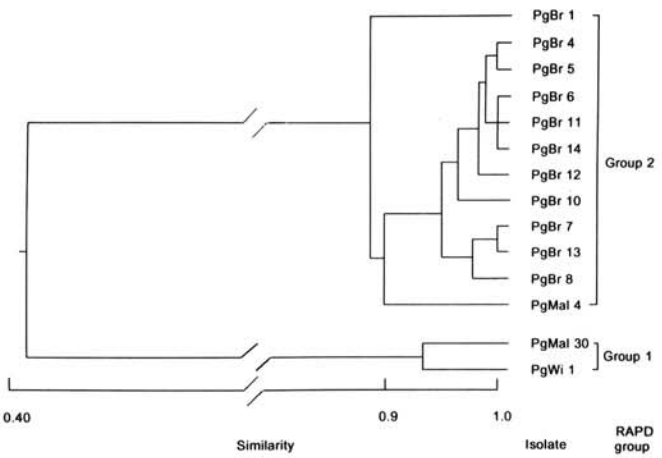

Fig. 5. Dendrogram generated based on random amplified polymorphic DNA (RAPD) fragments from 11 isolates of *Phaeoisariopsis griseola* from Brazil, one group 1 (Andean) isolate from Malawi (PgMal 30), one group 1 (Andean) isolate from the United States (PgWi 1), and one group 2 (Mesoamerican) isolate from Malawi (PgMal 4).

of 5 when these isolates were inoculated onto Andean genotypes (Table 2). An analysis of variance showed a highly significant interaction ($P < 0.001$) between the host and pathogen gene pools. There also were differences in the amount of disease induced by isolates within the same *P. griseola* group (Table 2). For example, all but one of the Mesoamerican genotypes from Malawi tested in this study showed relatively high levels of resistance against PgMal 30, a group 1 (Andean) isolate. However, only cultivar Namajengo showed resistance against PgMal 38, another group 1 (Andean) isolate. The two CIAT materials, which are both of Mesoamerican origin, showed high levels of resistance to both PgMal 30 and PgMal 38.

DISCUSSION

Several arguments provide strong evidence for the existence of two groups of *P. griseola* isolates that have a distinct evolutionary origin. At the genotypic level, RAPD patterns revealed two clearly distinct *P. griseola* groups, which were designated group 1 and group 2. These two groups were correlated with the gene pool of the predominant common beans grown in each of the three countries from which the isolates were collected. For example, the majority of isolates from Malawi belonged to group 1 and most of these isolates were recovered from Andean bean genotypes, which is consistent with the predominantly Andean origin of bean cultivars in that country (17, this study). Likewise, all the isolates from Brazil belonged to group 2 and all were recovered from Mesoamerican cultivars, which is in agreement with the Mesoamerican origin of most Brazilian cultivars (18). The Wisconsin isolates belonged to group 1 and were all recovered from red kidney beans, a known Andean type (3,18). Thus, these molecular marker results confirm previous observations (6) based on isozyme analysis suggesting the existence of two groups of *P. griseola* isolates, and establish that these groups correlate with the gene pool of their respective bean host plants.

At the phenotypic level, a statistically significant interaction was observed for the bean host-ALS pathogen relationship. Group 1 isolates, from Andean bean host genotypes, were more pathogenic on Andean genotypes, whereas group 2 isolates, from Mesoamerican bean genotypes, were more pathogenic on Mesoamerican bean genotypes. Therefore, both the genotypic and phenotypic variation suggest that the genetic diversity in *P. griseola* parallels that of its bean host, supporting the previous hypothesis of Gepts and Bliss (14). We further suggest that group 1 *P. griseola* isolates may have originated in the Andes, whereas group 2 isolates may have originated in Mesoamerica.

The two gene pools of *P. vulgaris* predated the domestication process in this species (23,24) and could have arisen through geographic isolation following dissemination from a presumed ancestor located in Ecuador and northern Peru (J. Kami, V. Becerra Velásquez, D. Debouck, and P. Gepts, *unpublished data*). Our data suggest that during the divergence process taking place in the host, a similar divergence could have taken place in *P. griseola* leading to increased pathogenicity of the fungus on its respective bean gene pool materials. Whether these genetic changes resulted from selective effects exercised by the host or the pathogen or from other phenomena such as genetic drift cannot be determined at this stage. However, the pathogenic distinction between the two *P. griseola* groups does not appear to be ab-

TABLE 2. Disease reaction of Malawian bean cultivars/lines after inoculation with five *Phaeoisariopsis griseola* isolates

	P. griseola isolates				
	Group 1 (Andean)		Group 2 (Mesoamerican)		
Bean cultivar	PgMal 30	PgMal 38	PgMal 4	PgMal 7	PgBr 8
Controls					
A240 (M)[a]	2[b]	2	4	4	6
A286 (M)	2	2	3	4	6
Topcrop (A)	8	8	8	8	8
Sutter Pink (M)	8	8	8	8	8
Mesoamerican genotypes					
Namajengo	3	2	8	8	7
Kantsilo	2	6	7	8	8
14-5	2	6	8	7	7
1-1	2	7	7	7	7
6-5	2	8	8	7	8
13-3	6	7	8	8	7
Mean for Mesoamerican genotypes		4 (2.3)[c]		8 (0.5)	
Andean genotypes					
8-7	7	4	2	2	3
22-2	8	8	4	3	7
3-13	2	2	5	6	2
18-10	7	3	5	4	2
5-2	7	6	5	5	7
11-1	8	8	6	6	7
21-5	7	5	7	5	6
12-4	8	8	2	3	3
Sapelekedwa	7	7	7	3	5
25-2	7	5	5	3	5
Kamsama	7	8	6	4	6
Nasaka	7	7	6	6	6
Bwenzi Lawana	7	8	7	7	6
7-8	8	8	6	7	6
16-6	8	8	6	6	5
2-10	9	7	6	5	5
17-1	8	8	3	2	3
24-6	7	8	3	3	4
Mean for Andean genotypes		7 (1.7)		5 (1.6)	

[a] A = Andean; M = Mesoamerican.

[b] Each value represents the average of readings from three separate experiments with three plants scored for each experiment. Disease severity ratings were made with a 1 to 9 rating scale in which 1 represents an immune reaction and 9 represents extreme susceptibility (4).

[c] Standard error in parentheses.

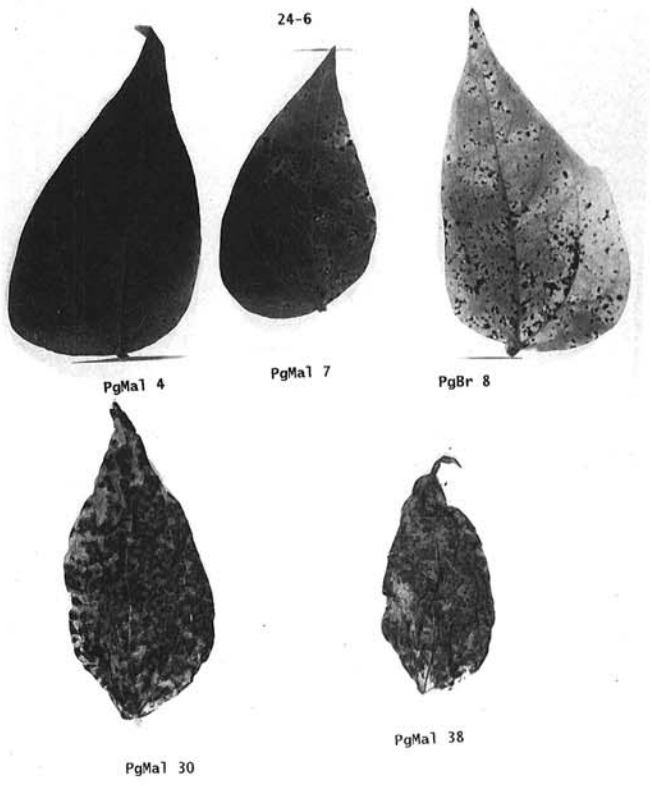

Fig. 6. Disease reaction of the Malawian cultivar 24-6 (Andean gene pool) after inoculation with *Phaeoisariopsis griseola* group 1 (Andean) isolates (PgMal 30 and PgMal 38) and *P. griseola* group 2 (Mesoamerican) isolates (PgMal 4, PgMal 7, and PgBr 8).

solute as bean genotypes in a certain gene pool are not uniformly resistant to all isolates of the same *P. griseola* group as shown by the comparison of inoculation results with group 1 isolates PgMal 30 and PgMal 38 on Mesoamerican cultivars (Table 2). This suggests possible hybridization(s) between host genotypes and/or pathogen isolates. Alternatively, independent mutations affecting host-pathogen relationships could have occurred in either the bean gene pools and/or *P. griseola* groups.

The two principal regions selected for our study, Malawi and Brazil, likely represent regions where selection and/or coevolution of *P. griseola* isolates has occurred as opposed to an accidental introduction event because materials of both gene pools can be found in these regions (although one of the *P. vulgaris* gene pools predominates in each region). A survey of phaseolin type showed that in Brazil, 57% of the traditional bean landraces analyzed had a Mesoamerican phaseolin type and 43% had an Andean type (17), although data from germ plasm explorations and from improved cultivar pedigrees show an increasing reliance on Mesoamerican bean types (40,41). A similar survey of Malawian bean landraces showed that 85% of the materials had an Andean phaseolin type and 15% had a Mesoamerican phaseolin type (15). Based on these results, we further hypothesized that if two corresponding groups of pathogen isolates existed, they would both exist in these countries together with their respective host groups. The coexistence of both host gene pools and pathogen groups was demonstrated in Malawi. In Brazil, insufficient sampling may account for the absence of Andean host germ plasm and group 1 (Andean) *P. griseola* isolates. Furthermore, results from the analysis of the Malawian materials show that it is possible to find group 2 (Mesoamerican) isolates on Andean host genotypes and vice versa. Thus, the host resistance to ALS detected in this study appears to be partial rather than absolute. The actual genetic control, whether a multigenic horizontal resistance or an oligogenic vertical resistance, remains to be verified by a genetic analysis in segregating progenies.

Our results have important implications regarding breeding for ALS resistance. First, it is possible to predict the prevalent *P. griseola* group in a certain bean-growing region based on the prevalent bean genotypes grown in that region and/or RAPD analysis of representative fungal isolates from that region. In areas where both Mesoamerican and Andean genotypes are grown, it will be interesting to study the evolution of the fungus. Perhaps, hybrid isolates between groups 1 and 2 might be detected. Although there is no known sexual stage for *P. griseola*, the presence of hybrid isolates would suggest the existence of a mechanism of recombination, perhaps parasexuality. Second, it becomes possible to choose, and more importantly to limit, the number of isolates needed to identify bean germ plasm with broad spectrum resistance to *P. griseola*. Using RAPD markers, *P. griseola* isolates can be classified into group 1 (Andean) and group 2 (Mesoamerican), and a few representative isolates can be selected for resistance screening. In the absence of such a way of classifying isolates a breeder would have to sample a much larger number of isolates to assure a broad representation of the genetic diversity of the pathogen. Third, in regions where group 1 (Andean) isolates predominate, Mesoamerican bean genotypes may provide effective sources of ALS resistance, and vice versa in regions where group 2 (Mesoamerican) isolates predominate. For example, in Malawi, where group 1 (Andean) isolates predominate, Mesoamerican genotypes such as the A 240 and A 286 lines may be useful donors of ALS resistance. Fourth, local germ plasm can occasionally provide a source of resistance. In Malawi, the Mesoamerican cultivar Namajengo may provide a useful source of resistance against group 1 (Andean) isolates, whereas Andean lines 12-4 and 17-1 may provide useful sources of resistance against group 2 (Mesoamerican) isolates. Local sources of resistant germ plasm may be particularly useful because these are presumably already adapted to local conditions and represent bean types preferred by local farmers and consumers.

Further studies of the variability of *P. griseola* isolates would allow the characterization of the occurrence (frequency and prevalence) of the two *P. griseola* groups in different bean regions, as has been done with *Leptosphaeria maculans* in crucifers (19) and *Magnaporthe grisea* in rice (27). Additionally, gene flow and seasonal variation of isolates during the growing season of the crop could also be analyzed. We are developing *P. griseola* group-specific PCR primers that could be used to rapidly detect *P. griseola* groups and serve as tools to further study the coevolution between the two organisms.

In conclusion, we have provided evidence suggesting a parallel organization in genetic diversity between the bean host and a fungal pathogen that presumably arose through coevolution of the two organisms. Our findings may have important consequences for disease resistance breeding in beans. Similar studies with other pathogens (e.g., *Colletotrichum lindemuthianum*, causal agent of anthracnose in beans) (26) or in other crops may reveal similar parallelism in the organization of genetic diversity of the host and associated organisms as suggested earlier by Gepts and Bliss (14).

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