Reactions of Selected Bean Accessions to Infection by *Macrophomina phaseolina*

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ABSTRACT

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Fifty-three bean (*Phaseolus vulgaris*) accessions were evaluated for their reactions to infection by *Macrophomina phaseolina* under field and greenhouse conditions. A field nursery was established on an experimental farm near Santander de Quilichao, Colombia. All accessions were evaluated in paired rows, with and without inoculation, arranged in a randomized block design with three replicates. Field inoculations were made by placing 4 g of whole rice seeds colonized by *M. phaseolina* per 2-m row on top of the bean seeds in the open furrow. The accessions that showed resistant and intermediate reactions to *M. phaseolina* and appropriate controls totaling 36 entries were reevaluated in a similar nursery on the same farm. The materials included in the second nursery plus other accessions for a total of 49 also were evaluated in the greenhouse. Bean seeds were covered with 150 ml (layer 2–3 cm) of pasteurized soil infested with dried sclerotia of *M. phaseolina* (2 g/kg soil). Results of the field evaluation were similar to those obtained from the greenhouse. Twenty-two and 15 accessions were classified as resistant (disease severity rating [DSR] = 1–3) and intermediate (DSR = 3.1–6.0), respectively, following the CIAT evaluation scale of 1 (no visible symptoms) to 9 (plants dead). Among the highly resistant accessions were A 300, BAT 85, BAT 332, BAT 477, BAT 1385, BAT 1651, IPA 1, San Cristobal 83, EMP 86, and G 5059 (H6 Mulatinho). A 70, A 464, A 294, Rio Tibagi, and ICA Pijao were among the most susceptible to *M. phaseolina*. Based on these data, an international bean nursery consisting of 40 accessions was established and is available for evaluation against *M. phaseolina* in different bean production areas.

Macrophomina phaseolina (Tassi) Goid. incites charcoal rot on many agronomic crops including beans (*Phaseolus vulgaris* L.) (2,6,10). This disease has been reported on beans in many countries of the Americas and Africa. Damage to beans by *M. phaseolina* and epiphytotic development of charcoal rot appears to be most severe at high temperatures during periods of drought (3,4,6,8). Losses in beans caused by *M. phaseolina* are due to preemergence and postemergence death of seedlings or reduced vigor and yield of older plants. Severely infected plants show wilting, chlorosis, premature defoliation, and early maturity or death. Data from drought and disease evaluation nurseries suggested that cultivars identified at CIAT as drought-tolerant also possess resistance to *M. phaseolina* (3).

Abawi and Pastor-Corrales (1) evaluated the efficiency of several inoculum preparations and screening procedures for determining the reactions of bean germ plasm accessions to infection by *M. phaseolina*. The most effective methodology in greenhouse tests was to cover bean seeds with 2–3 cm of soil artificially infested with *M. phaseolina* (2 g of dry sclerotia per kilogram of pasteurized soil). Whole rice seeds colonized with *M. phaseolina* also were highly efficient in causing charcoal rot, and the resulting symptoms permitted the differentiation of susceptible and resistant bean germ plasm. Large volumes of the colonized rice seeds can be produced easily and rapidly, and thus, this inoculum is suitable for extensive field evaluation trials. The same study also showed that isolates of *M. phaseolina* obtained from infected bean tissues varied in their virulence on the susceptible cultivar California Light Red Kidney and the breeding line A 70 but not on the resistant breeding line BAT 477. The objective of this study was to determine the reactions of selected dry edible bean germ plasm accessions to infection by *M. phaseolina*. A brief summary of these results was published previously (9).

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MATERIALS AND METHODS

A highly virulent isolate (isolate 34) of *M. phaseolina* obtained from an infected Chileno bean plant near Palmira, Colombia, was used throughout this study. Dry sclerotia and colonized whole rice seeds were prepared as described previously (1) and used in greenhouse and field screening tests, respectively.

Field tests. The first evaluation trial contained 53 accessions and was established in a field with a history of high incidence of charcoal rot on the CIAT's Quilichao substation. The farm is located at an altitude of 990 m, has an average temperature of 23.8 C (29.5 C maximum and 18.3 C minimum), and precipitation of 1,757 mm/yr. The soil of the trial field was loam with a pH of 4.5–5.5 and 6–8% organic matter. The plot area was plowed and prepared with tractor-mounted equipment but was marked and planted manually. The trial was arranged in a randomized block design with three replicates. Each plot consisted of two rows 2 m long. Planting furrows were opened manually, and a complete fertilizer (10-30-10 NPK) was applied at 300 kg/ha. Carbofuran (Furadan) was mixed with the fertilizer at 1 kg/ha for soil insect control. The opened furrows were scratched with a hoe to lightly incorporate the fertilizer and insecticide. Seeds of each accession were planted with 30 seeds per 2-m row. One row then was inoculated with rice seeds colonized by *M. phaseolina*, about 4 g/2-m row (two or three colonized rice seeds per bean seed). The rows were covered, and the plot area was immediately sprayed with a mixture of the herbicides linuron (Afalon), paraquat (Gramoxone), and pendimethalin (Prowl) at 1, 3, and 2 L, respectively, in 200 L of water per hectare. The trial was maintained according to commercial production recommendations including the foliar application of fertilizer and micronutrients, disease and insect control, and weeding. The plots were irrigated as needed. Total number of emerged plants and the number of plants infected with *M. phaseolina* were recorded 3 wk after planting. Number of surviving plants, number infected with *M. phaseolina*, and seed weight were recorded at harvest. A second nursery consisting of 36 accessions selected from the first trial was established similarly and evaluated. The first and second nurseries were planted on 26 September and 26 November 1985, respectively.

Greenhouse tests. Dry sclerotia of *M. phaseolina* were produced on the synthetic liquid medium as described previously (1). Sclerotia were mixed thoroughly in pasteurized soil at a rate of 2 g/kg of soil. About 150 ml of the infested soil was placed on top of bean seeds planted in 10-cm pots (four seeds per pot), forming a layer about 2–3 cm over the seeds. The pots were then incubated in a greenhouse at 20–33 C and 35–80% relative humidity for 2 wk. Forty-nine accessions were evaluated and arranged in a randomized block design with four replicates. Selection of accessions included was based on the results of the field trials of this study, tropical adaptation, and use in the breeding program at CIAT as parental lines. Disease severity ratings were recorded 2 wk after planting on a scale of 1 (no visible symptoms) to 9 (all stem tissues and growing tip affected, plants dead) (1,5).

Data from each test were subjected to analysis of variance, and LSDs were calculated if *F*-tests indicated statistical significance.

Table 1. Evaluation of bean accessions for resistance to *Macrophomina phaseolina* in a field near Quilichao, Colombia, in 1985–1986

Parameter measured	*M. phaseolina* inoculum[a]	Parameter measured: Range	Av.	$LSD_{0.05}$
First nursery (53 accessions)				
Emergence (no./2 m)	−	3.7–22.3	14.6	7.07
	+	0.0–16.0	8.9	7.27
Plants at harvest (no./2 m)	−	2.3–20.0	13.5	7.49
	+	0.0–14.3	6.1	5.99
Seed wt (g/2 m)	−	32.7–448.7	174.2	118.31
	+	0.0–240.3	94.9	93.78
M. phaseolina infections (no. plants/2 m)				
At emergence	−	0.0–1.7	0.4	1.18
	+	0.0–6.3	2.7	3.48
At harvest	−	0.0–1.7	0.4	0.99
	+	0.0–2.7	0.3	1.26
Second nursery (36 accessions)				
Emergence (no./1 m)				
	−	5.5–15.0	12.1	4.21
Plants at harvest (no./1 m)	+	0.0–14.0	10.2	5.08
	−	4.0–14.0	9.9	5.19
Seed wt (g/m)	+	1.5–12.0	6.6	5.29
	−	1.5–117.0	52.2	53.92
M. phaseolina infections	+	9.5–99.0	46.7	51.68
(no. plants/1 m)				
At emergence	−	0.0–0.5	0.1	0.50
	+	0.0–7.5	2.7	3.95
At harvest	−	1.0–10.0	4.2	5.49
	+	0.5–8.0	3.2	4.46

[a] Inoculum consisted of whole rice seeds colonized by *M. phaseolina* placed in the seed furrow at a rate of 4 g/2-m row (two or three colonized rice seeds per bean seed).

Table 2. Growth parameters of selected bean accessions evaluated for their reactions to *Macrophomina phaseolina* under field conditions near Santander de Quilichao, Colombia

	Emergence/2 m[b]		Stand/2 m[b]		*M. phaseolina*-infected plants/2 m[b]		Seed wt.[b] (g/2 m)	
Bean accessions[a]	Inoc.[c]	Uninoc.[c]	Inoc.	Uninoc.	Inoc.	Uninoc.	Inoc.	Uninoc.
BAT 477	16.0	18.7	14.3	19.0	2.0	0.3	234.3	448.7
A 300	15.7	22.3	11.0	20.0	3.3	0.3	240.3	305.3
V 8017	14.0	20.0	10.3	18.0	3.0	0.0	238.7	103.7
CG 82-67	14.0	20.0	12.7	19.7	0.7	0.0	28.7	88.3
BAT 1293	13.7	12.7	6.0	9.7	6.3	1.3	119.3	178.7
BAT 125	13.3	22.3	10.0	20.0	2.7	0.3	164.3	279.7
CG 82-121	13.3	15.7	9.0	14.0	1.3	0.0	91.7	61.7
BAT 332	12.7	17.7	8.7	17.3	3.7	0.0	188.3	293.0
BAT 1232	12.3	11.3	5.7	10.3	6.3	0.0	132.3	217.0
Aroana 80	12.3	17.7	8.0	15.0	4.3	1.0	130.7	189.7
San Cristobal 83	12.3	14.7	8.3	12.7	2.0	1.0	73.7	183.7
BAT 1385	10.3	17.0	6.3	16.7	3.7	0.0	132.7	185.7
BAT 1289	10.3	11.7	4.3	9.7	5.7	1.3	164.7	195.3
G 5059	10.3	18.0	6.7	17.3	0.7	0.3	75.3	237.7
BAT 1651	9.7	19.7	5.0	18.3	2.7	0.0	60.3	275.0
BAT 1400	9.0	15.3	7.3	12.7	1.3	0.0	156.3	176.7
BAT 868	9.0	10.7	6.7	10.0	3.0	0.3	202.0	225.7
A 464	6.3	11.3	2.7	9.3	3.3	1.0	11.0	109.7
Sanilac	2.3	12.0	1.7	8.3	0.3	0.3	0.0	82.3
BAT 1592	0.3	7.0	0.0	5.0	0.0	0.7	0.0	38.7
$LSD_{0.05}$[d]	7.3	7.1	6.0	7.5	3.5	1.2	93.8	118.3

[a] All accessions can be obtained as designated from CIAT, Cali, Colombia.
[b] Emergence and infected plants were recorded 3 wk after planting; stand and seed wt were recorded at normal harvest time.
[c] *M. phaseolina*-inoculated (4 g of colonized whole rice seeds per 2-m row) or uninoculated plots.
[d] Least significant differences given are those for the entire test, which included 53 bean accessions.

RESULTS

Field evaluation. The 53 and 36 bean accessions evaluated in the first and second field nurseries, respectively, varied in their reactions to *M. phaseolina* under both natural and artificial soil infestations (Tables 1 and 2). Significant differences ($P = 0.05$) were detected among the accessions in emergence count, number of plants at harvest, seed yield, and incidence of charcoal rot. However, charcoal rot incidence and damage was generally higher in the artificially infested plots (Tables 1 and 2). Also, disease pressure was higher in the second nursery than in the first as the data on seed yield and disease incidence indicate (Table 1). Emergence and stand count at harvest were most indicative of the overall reaction of bean germ plasm to infection by *M. phaseolina*. For example, BAT 477 (highly resistant to *M. phaseolina*) had an emergence and stand at harvest of 16.0 and 14.3 plants per 2-m row, respectively, in infested plots of the first nursery. The comparable counts for the susceptible accession A 464 were 6.3 and 2.7, respectively (Table 2). The accessions that performed well in the first and/or second evaluation nursery were considered highly resistant to *M. phaseolina* under field conditions and included BAT 332, BAT 477, BAT 868, BAT 1385, BAT 1581, A 120, A 300, G 5059 (H6 Mulatinho), Aroana 80, San Cristobal 83, PI 136738, and IPA 1. In contrast, A 464, Sanilac, BAT 1210, BAT 1360, and BAT 1592 were among the accessions with the poorest performance.

Greenhouse evaluations. The reactions of the 49 accessions included in the test after 2 wk of incubation are listed in Table 3. BAT 85, BAT 332, BAT 477, BAT 868, BAT 1289, BAT 1385, BAT 1400, BAT 1651, V 8025, EMP 86, CG/82-24, CG/82-115, IPA 1, G 5059 (H6 Mulatinho), and San Cristobal 83 were the most resistant to *M. phaseolina*. A 70, A 294, A 464, ICA Pijao, and Rio Tibagi were the most susceptible. These results suggest that a close correlation exists between the field performance of the selected bean accessions under high incidence of charcoal rot and their reactions as determined by the greenhouse screening procedure. Based on the greenhouse and field data collected, an international nursery consisting of 40 accessions is now available from CIAT for testing against infections by *M. phaseolina* in different bean-growing areas.

DISCUSSION

Several bean accessions were found

highly resistant to infection by *M. phaseolina*. All accessions with a high level of resistance to *M. phaseolina* in field nurseries also were resistant in the greenhouse evaluation. These results further substantiate the accuracy and efficiency of the dry sclerotial inoculum and the screening procedure for *M. phaseolina* as reported previously (1). The field evaluation procedure was effective in separating the reactions of bean accessions into general groups as promising materials for further evaluation and highly susceptible accessions. However, there was considerable variability in disease incidence early in the growing season that was probably due to manner of placement of the inoculum of *M. phaseolina* in the row. Whole rice seeds colonized by *M. phaseolina* must be in contact with bean seeds for infection to occur under field conditions. In addition, the reactions of bean accessions that are not adapted to the test location and other prevailing stress factors should be assessed carefully.

The reactions of susceptible cultivars were evident shortly after planting. The first indication of susceptibility was the poor emergence of the highly susceptible cultivars such as A 464, A 70, Sanilac, and BAT 1360. In addition, emerged seedlings of these accessions generally showed the characteristic dark sunken lesions on the cotyledon tissues. These lesions expanded rather rapidly, reaching the stem tissues within 3–5 days and eventually killing the seedling within 2 wk. These symptoms occurred on susceptible accessions both in the field and under greenhouse conditions. These observations agree with those of Gangopadhyay et al (7), who reported that *M. phaseolina* causes severe postemergence damping-off of soybean seedlings with plant losses of up to 77%. Secondary infections resulting from the expansion of infections on lower stem tissues below the soil level or those initiated by airborne inoculum appear to occur later in the growing season, especially after plants start to senesce. At harvest, high incidence of charcoal rot was observed even on the resistant plants. These late infections increased after the plants were stressed by high temperatures and dry periods. Infections of beans by *M. phaseolina* have been reported to be most severe under high temperatures and drought stresses (2,3,6,8).

The reactions of many of the bean accessions reported here are in general agreement with the observations made by CIAT scientists in the drought-tolerance nurseries conducted in several locations over many seasons where charcoal rot has occurred in epidemic proportions (3,4). The cultivar Negrito has been reported in the literature as immune to *M. phaseolina* in Colombia (11). The most interesting finding of our study is that many of the bean cultivars identified by CIAT scientists as drought-tolerant, such as BAT 477 and H6 Mulatinho (4), also were highly resistant to *M. phaseolina*. In contrast, all known drought-susceptible entries, such as A 70 and BAT 1224, were susceptible to *M. phaseolina*. This apparent relationship or the suggestion that the mechanism of drought tolerance also confers resistance to *M. phaseolina* under field conditions may be proven incorrect if a larger number of bean accessions, selected randomly, are evaluated against *M. phaseolina*. However, if this relationship exists, it will have significant implications for research dealing with the identification of drought-tolerant bean accessions. Determining the reactions of bean accessions to *M. phaseolina* with the dry sclerotial inoculum is easy, rapid, and accurate and thus will facilitate the difficult task of documenting drought tolerance in a large number of parental germ plasm accessions. Resistance to *M. phaseolina* may be needed for the expression of drought tolerance in bean germ plasm grown in areas infested with *M. phaseolina*. Furthermore, it is important to elucidate the effect of soil moisture deficit on the severity and damage of *M. phaseolina* to beans.

Table 3. Reactions of bean accessions to *Macrophomina phaseolina* (isolate 34) under greenhouse conditions

Bean accession[a]	DSR (1-9)[b]	Bean accession	DSR (1-9)
A 83	6.8[c]	BAT 1477	4.9
A 55	5.5	BAT 1500	3.7
A 70	7.3	BAT 1581	2.7
A 120	3.0	BAT 1616	3.1
A 247	6.8	BAT 1651	1.0
A 249	5.5	BAT 1669	3.4
A 252	4.5	RIZ 30	6.2
A 281	4.8	V 8010	3.3
A 294	7.8	V 8025	1.6
A 295	4.0	EMP 86	1.4
A 464 (suscep. check)	7.7	CG/82-84	1.0
BAT 85	1.1	CG/82-67	5.7
BAT 25	5.8	CG/82-69	2.8
BAT 332	1.0	CG/82-70	4.4
BAT 336	4.1	CG/82-79	2.9
BAT 477 (resist. check)	1.3	CG/82-106	3.2
BAT 868	1.9	CG/82-115	1.7
BAT 1224	4.7	CG/82-121	1.5
BAT 1232	2.2	Aroana 80	3.3
BAT 1289	1.2	ICA Pijao	7.4
BAT 1293	2.8	IPA 1	1.3
BAT 1297	2.3	San Cristobal 83	1.0
BAT 1375	3.5	Rio Tibagi	7.5
BAT 1385	1.0	G 5059 (H6 Mulatinho)	1.0
BAT 1400	1.4		
		$LSD_{0.05}$	2.12

[a] All accessions can be obtained as designated from the germ plasm bank, the bean breeders, or the bean pathologist at CIAT, Cali, Colombia.

[b] Disease severity ratings were recorded 14 days after inoculation using the CIAT evaluation scale (9) of 1 (no visible symptoms) to 9 (all stem tissues and growing tip affected, plants dead). Inoculation procedure consisted of covering seeds with a layer (2–3 cm) of pasteurized soil infested with 2 g of sclerotia of *M. phaseolina* per kilogram of soil. *M. phaseolina* isolate 34 was obtained from infected bean cultivar Chileano plant near Palmira, Colombia.

[c] Each number is an average of four replicates with four seeds per replicate (10-cm pot).

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