Inheritance of the Resistance to *Macrophomina phaseolina* and Identification of RAPD Markers Linked to the Resistance Genes in Beans

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ABSTRACT

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The inheritance of resistance to *Macrophomina phaseolina* was determined using traditional approaches and molecular markers. Inheritance studies were based on a cross between the resistant accession BAT-477 and the susceptible accession A-70. Resistance to *M. phaseolina* was examined by inoculating bean seeds with soil infested with sclerotia of *M. phaseolina*. The ratio of resistant to susceptible plants in the F_2 population was close to 9:7. Bulked segregant analysis identified two random amplified polymorphic DNA (RAPD) markers linked to *M. phaseolina* resistance. The first RAPD marker ($B459_{1600}$) was a 1.6-kb band present in the susceptible and absent in the resistant plants. The second RAPD marker ($B386_{900}$) was a 0.9-kb band present in the resistant, but absent in the susceptible plants. Each of the RAPD markers segregated in a 3:1 ratio as expected, and the segregation of both markers followed a 9:3:3:1 ratio expected for two unlinked loci. In 15 of the 16 F_3 families examined, the genotype of the markers correctly predicted the genotype (heterozygous or homozygous) for disease resistance. The one exception could be attributed to recombination between $B459_{1600}$ and the linked susceptible gene. These data were consistent with the hypothesis that, in BAT-477, resistance to *M. phaseolina* is controlled by two dominant complementary genes. The symbols *Mp-1* and *Mp-2 (Macrophomina phaseolina)* were proposed for these genes.

Additional keywords: ashy stem blight, charcoal rot, *Phaseolus vulgaris*, polymerase chain reaction.

Charcoal rot or ashy stem blight of common beans (*Phaseolus vulgaris* L.) incited by the fungus *Macrophomina phaseolina* (Tassi) Goidanich (synonym *Rhizoctonia bataticola* (Taubenhaus) E. J. Butler) (9,23) causes considerable damage in bean production areas that are characterized by high temperature and drought conditions. *M. phaseolina* is a deuteromycetous soilborne fungus. The fungus is found mostly in an anamorphic stage either as microsclerotia or pycnidia. In the latter stage, *M. phaseolina* is assigned to the form-class Coelomycetes (13).

M. phaseolina has a wide host range and geographic distribution and is also a major pathogen of many crops including sorghum, cotton, soybean, chickpea, sunflower, corn, cowpea, and peanut (13,23). The fungus survives in the soil mainly in the form of sclerotia, the primary source of inoculum (4), and is also seedborne (2). The fungus can grow, produce high quantities of sclerotia, and survive and infect beans under relatively dry conditions (18,19). *M. phaseolina* has been recognized as a drought-favored pathogen.

M. phaseolina infects bean plants at all stages. Damage can result in poor seedling establishment, preemergence and postemergence damping-off, and reduced vigor and productivity of older plants. Major symptoms on infected plants are stunting, chlorosis, premature defoliation, early maturity, and death. In addition, this fungus readily infects cotyledons, stem, pod tissues, and seeds (1,9,21). Resistance in beans to *M. phaseolina* has been observed to be associated with drought tolerance (21). However, this observation requires further documentation.

The identification of bean germ plasm with resistance to *M. phaseolina* and the development of resistant and adapted cultivars is an appropriate and lasting measure of control for charcoal rot (1). Bean cultivars that are highly resistant to *M. phaseolina* and adapted to the different production areas with acceptable agronomic characteristics are not available. The reactions of selected bean accessions to *M. phaseolina* have already been identified (5,21), but little information is available regarding the genetic basis of resistance in beans to *M. phaseolina*. Miklas and Beaver (14) determined that field resistance to *M. phaseolina* is controlled by more than one gene. The apparent polygenic basis of *M. phaseolina* resistance in beans, combined with the difficulties in working with soilborne diseases, has impeded progress in developing resistant germ plasm and cultivars.

Restriction fragment length polymorphism (RFLP) and random amplified polymorphic DNA (RAPD) linkage maps of beans have been developed by Nodari et al. (16,17), Zhu and Weeden (27), and Vallejos et al. (25). The genetic map of bean is being used for studies on evolution, breeding strategies, and tagging of genes of resistance to insect pests and diseases. An early generation screening can be developed using DNA molecular markers (11,22,24). In beans, RFLP or RAPD markers have been identified to be linked to the following disease resistance genes: rust (8,15), common bacterial blight (17), and anthracnose (3). The objectives of this study were to investigate the genetic basis of the resistance to *M. phaseolina* in selected bean lines and to identify RAPD marker(s) linked to *M. phaseolina* resistance in beans.

MATERIALS AND METHODS

Inheritance of the resistance. Inheritance studies were performed on F_2 or F_3 progeny of crosses between the resistant accession BAT-477 and the susceptible accession A-70, previously characterized by Pastor-Corrales and Abawi (21). Seeds were

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obtained from the International Center for Tropical Agriculture, CIAT, located in Palmira, Colombia. An isolate of *M. phaseolina* (Mp#50), obtained from naturally infected beans in Colombia, was used throughout this study. This isolate was stored in dried, colonized table beet seeds at 5°C (18) and transferred to potato-dextrose agar as needed. Sclerotia were produced by growing *M. phaseolina* in potato-dextrose broth. Sclerotia were collected and dried from colonies grown for 15 days at 30°C. Dried sclerotial masses were ground in a mortar (18).

One hundred fifteen F_2 plants (designated as population A) were tested for resistance to *M. phaseolina* by covering the seeds with a layer of soil (150 ml/10-cm pot) infested with sclerotia of *M. phaseolina* (2 g/kg of soil) and incubated in growth chambers with alternating 12-h periods of light and dark at 25°C. This inoculation procedure was similar to that reported by Abawi and Pastor-Corrales (1), except that a modification was made that reduced the percentage of escapes. Instead of placing the entire soil inoculum on top of the bean seeds, a 1-cm-thick layer (50 ml) was added above 200 ml of pasteurized soil (about 4 to 5 cm) in 10-cm clay pots. Two seeds were placed on top of this layer and then covered with a layer of 100 ml of inoculum (about 2 cm). Pots were incubated for 7 days in the growth chamber at 25°C and then transferred to a greenhouse at 25 to 30°C for 2 to 3 weeks. Disease severity ratings were recorded at weekly intervals using a pretransformed scale of 1 to 9, in which 1 referred to no visible symptoms and a rating of 9 indicated that all stem tissues and the growing point were affected (1). Plants scored 1 to 3 were considered resistant; plants scored 4 to 9 were considered susceptible.

Identification of RAPD marker(s) linked to *M. phaseolina* resistance in beans. An F_2 population of 79 individuals (designated as population B) from the BAT-477 × A-70 cross was grown to maturity in the greenhouse. Tissue from each of the F_2 plants, as well as from the two parent accessions, was collected and the DNA extracted. Five to ten F_3 plants derived from each of the F_2 plants were screened for *M. phaseolina* reaction to determine the genotype of the F_2 plants (progeny test). DNA was isolated by the method described by Lodhi et al. (10). Bulk segregant analysis (12) was used to identify RAPD marker(s) linked to *M. phaseolina* resistance. Resistant and susceptible bulks were generated using the DNA from the F_2 plants of six F_3 families in which all members were resistant or susceptible, respectively.

Polymerase chain reaction (PCR) amplifications were performed for each primer using DNA from the bulks and each parent as templates. Primers were obtained mainly from the Biotechnology Center of the University of British Columbia (Vancouver, Canada) and from Operon Technologies, Inc. (Alameda, CA). Amplification reactions contained 20 ng of genomic DNA, 2.5 µl of 10× buffer, 1.5 µl of $MgCl_2$, 0.13 mM of each of the four dNTP, 0.2 mM oligonucleotide primer, 0.5 unit of *Taq* polymerase (Promega Corp., Madison, WI), and 18 µl of sterilized distilled water (total volume: 25 µl). The reaction mix was overlaid with a drop (about 20 µl) of mineral oil. PCR reactions were carried out using a thermocycler (MJ Research Inc., San Francisco, CA) with the following profile: i) 94°C for 2 min, ii) 35°C for 2 min, and iii) 72°C for 2 min. The total number of cycles was 40, the final step was at 72°C for 8 min, and the reactions were held at 6°C until electrophoresis. PCR products were analyzed in 1% NuSieve (FMC BioProducts, Rockland, ME):1% agarose (International Biotechnologies, Inc., Rochester, NY). Electrophoresis was run for 4 h at 8 V/cm, and gels were stained with ethidium bromide (0.01 mg/ml) for 15 min and photographed over a 302 nM UV light. Cosegregation of the identified marker and the resistance phenotype was confirmed by analyzing the marker against the parents and the segregating population.

In another test, 181 F_2 plants derived from a cross between the same parental bean lines (BAT-477 × A-70) were divided into two groups. The first group (110 plants designated as population C-1) was inoculated with *M. phaseolina* and the reaction of the plants was scored. DNA was extracted from only the resistant plants (plants that survived the inoculation), and the segregation ratio of the RAPD marker was determined. Seeds from the second group (71 plants designated as population C-2) were planted and grown in the greenhouse and were not inoculated. DNA from tissue collected from these plants was isolated to determine the segregation ratio of the identified RAPD marker in an unselected population. A chi-square test was used to confirm simple dominant inheritance of the resistance phenotype and the identified RAPD marker.

Sixteen F_3 families derived from 16 F_2 plants resistant to *M. phaseolina* were also included in this study (population D). The phenotype of the F_2 plants was identified by inoculating 10 F_3 plants derived from each F_2 plant with *M. phaseolina* using the method described previously. The segregation of the identified marker(s) linked to the resistance gene(s) was also determined in these populations.

RESULTS

Inheritance of the resistance. From the F_2 population (population A) of 115 plants (BAT-477 × A-70), 62 were scored as resistant and 53 as susceptible. BAT-477 remained resistant and A-70 was severely infected (average disease severity rating = 1.5 and 9, respectively). F_1 plants were resistant to *M. phaseolina* (average disease severity rating = 2). A high percentage of the F_2 plants appeared to display parental phenotypes (Fig. 1). Reaction of the F_2 plants resistant to *M. phaseolina* was generally scored as 1 or 2. Susceptible F_2 plants were generally scored from 6 to 9 (Fig. 1). The number of resistant plants with a disease severity rating of 3 was five. Few plants were scored as 4 or 5 (only four plants for each rating) (Fig. 1). Plants with scores of 1 to 3 were considered resistant, and plants with scores of 4 to 9 were considered susceptible. Plant reactions scored as 3 referred to development of lesions that were limited to the cotyledonary tissues and showed very restricted lesions on the stems of about 1 to 2 mm. Plant reactions scored as 4 indicated that a lesion from cotyledonary tissue had already reached the stem and had produced lesions of about 1 cm on the stem. Plants with lesions that had progressed more than 2 cm on the stem were scored as 5.

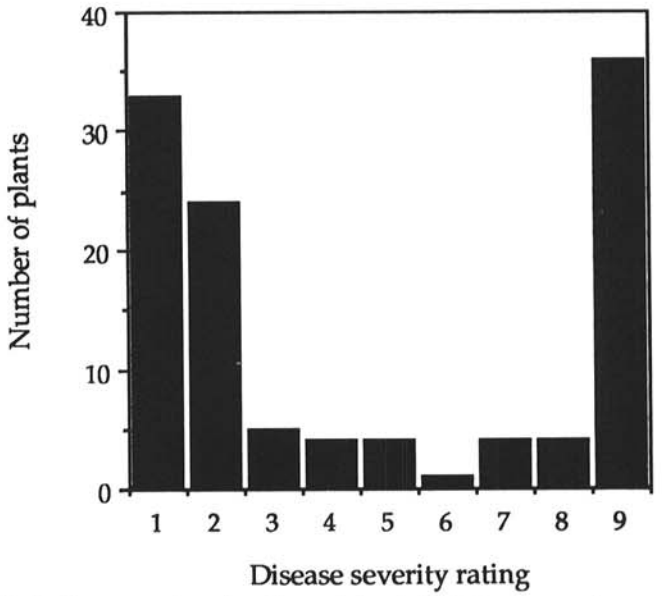

Fig. 1. Disease reaction of an F_2 population derived from a cross between bean lines BAT-477 and A-70 that are resistant and susceptible, respectively, to *Macrophomina phaseolina*. Disease severity was recorded using a pretransformed scale of 1 to 9, in which 1 referred to no visible symptoms and a rating of 9 indicated that all stem tissues and the growing point were affected. Plants scored 1 to 3 were considered resistant; those scored 4 to 9 were considered susceptible.

TABLE 1. Resistant (R) and susceptible (S) segregation ratios of 115 F_2 plants (population A) and 110 selected F_2 plants (population C-1) derived from a cross between bean lines BAT-477 and A-70 that are resistant and susceptible, respectively, to *Macrophomina phaseolina*

	Number of plants		Expected ratio		
	R	S	R:S	X^2	*P* value
Population A					
Observed	62	53			
Expected	64.7	50.3	9:7	0.25	0.633
Expected	86	29	3:1	26.55	<0.001
Expected	93.4	21.6	13:3	56.19	<0.001
Expected	107.8	7.2	15:1	310.79	<0.001
Population C-1					
Observed	68	42			
Expected	61.9	48.1	9:7	1.33	0.254
Expected	82	28	3:1	5.79	0.016
Expected	89.4	20.6	13:3	27.35	<0.001
Expected	103.1	6.9	15:1	190.50	<0.001

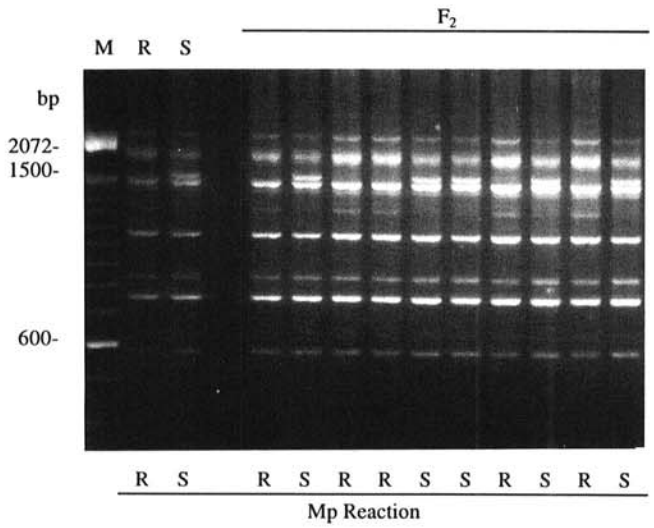

Fig. 2. Segregation of RAPD marker $B459_{1600}$ in an F_2 population derived from a cross between bean lines BAT-477 and A-70 that are resistant and susceptible, respectively, to *Macrophomina phaseolina*. $B459_{1600}$ detected a band present in the susceptible and absent in the resistant plants.

The result obtained, showing that most of the F_2 plants exhibited parental phenotypes, suggested that few genes were involved in conferring resistance to *M. phaseolina*. Different segregation ratios for the interaction of few genes in the resistance were analyzed (Table 1). Among the ratios tested, the resistant to susceptible ratio of 9:7 was the only one that fit the data. These results were consistent with the hypothesis that resistance to *M. phaseolina* in beans is controlled by two dominant complementary genes. The 110 F_2 plants in population C-1 segregated 68:42 for resistance and susceptibility. The segregation was consistent with the 9:7 expected ratio (Table 1). Thus, the symbols *Mp-1* and *Mp-2 (Macrophomina phaseolina)* were proposed for these genes.

Identification of RAPD marker(s) linked to *M. phaseolina* resistance in beans. About 600 primers were tested, and two of them detected polymorphism between the resistant and susceptible bulk. The first primer displaying a difference between the two bulks was B459 (sequence 5′-GCAATCCCAC-3′). This primer amplified a fragment of 1.6 kb (designated $B459_{1600}$) that was present in the susceptible and absent in the resistant plants (Fig. 2). The second primer identified was B386 (sequence 5′-GGCTCACTAA-3′). This primer amplified a fragment of 0.9 kb (designated $B386_{900}$) that was present in the resistant, but absent in the susceptible plants (Fig. 3).

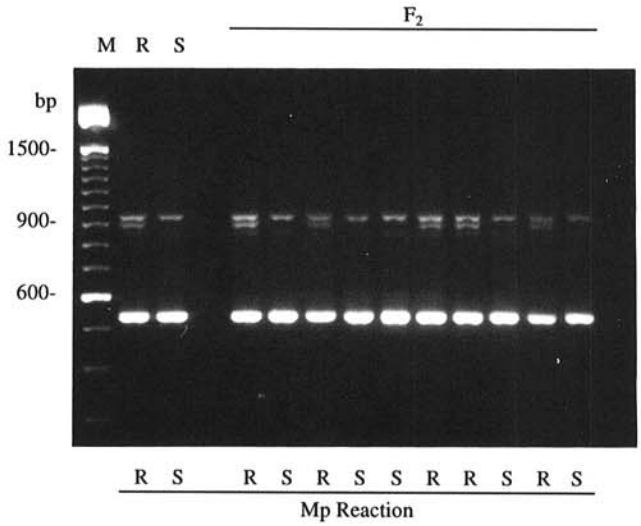

Fig. 3. Segregation of RAPD marker $B386_{900}$ in an F_2 population derived from a cross between bean lines BAT-477 and A-70 that are resistant and susceptible, respectively, to *Macrophomina phaseolina*. $B386_{900}$ detected a band present in the resistant, but absent in the susceptible plants.

TABLE 2. Segregation ratios of two RAPD markers in 71 F_2 plants (selected from an F_2 population composed of 181 seeds and designated as population C-2) obtained from a cross between bean lines BAT-477 and A-70 that are resistant and susceptible, respectively, to *Macrophomina phaseolina*[a]

	Band		Expected ratio		
Marker	Present (+)	Absent (–)	+:–	X^2	*P* value
Expected	53	18			
$B459_{1600}$	47	24	3:1	2.67	0.132
$B386_{900}$	56	15	3:1	0.66	0.383

[a] RAPD marker $B459_{1600}$ detected a band present (+) in the susceptible and absent (–) in the resistant plants. $B386_{900}$ detected a band present (+) in the resistant, but absent (–) in the susceptible plants.

The segregation of each of these two RAPD markers in the 71 F_2 uninoculated plants (population C-2) fit the 3:1 ratio expected for a dominant marker (Table 2). The two markers cosegregated in a 9:3:3:1 ratio, consistent with independent assortment (Table 3). Joint segregation analysis indicated that the two markers were not closely linked (six repulsion phase recombinants in the 71 plants assayed), and the joint segregation ratio of 38:18:9:6 did not differ significantly from that expected for independent assortment (Table 3).

The segregation ratios of the $B459_{1600}$ and $B386_{900}$ RAPD markers in the 68 F_2 resistant plants that survived *M. phaseolina* inoculation (population C-1) differed dramatically from the 3:1 ratio (Table 4). $B459_{1600}$ segregated in a ratio close to 2:1, and $B386_{900}$ was present in nearly all the surviving plants. These results indicated that each of the markers was linked to a gene involved in the expression of resistance. The segregation ratio of these two markers in populations C-1 and C-2 were the ratios that would be expected if the two markers were linked to two loci controlling the resistant phenotype, with $B459_{1600}$ being linked in coupling to the susceptible allele at one locus and $B386_{900}$ being linked in coupling to the resistant allele at the other locus.

Assuming no recombination, it was possible to predict the expected segregation ratios for these two markers in population C-1 (resistant plants that survived *M. phaseolina* inoculation). The marker $B386_{900}$, linked in coupling with one of the resistance genes, would have the expected ratio of 1:0 in the selected resistant population; and the marker $B459_{1600}$, in repulsion phase with the other gene, would have an expected segregation ratio of 2:1. These expected ratios fit the ratios observed (Table 4). The overall

TABLE 3. Goodness-of-fit for a 9:3:3:1 segregation ratio of the two RAPD markers linked to *Macrophomina phaseolina* resistance genes in an F_2 population from the cross between bean lines BAT-477 (resistant) and A-70 (susceptible)[a]

	Band present (+)/Band absent (–)						
	+/+	+/–	–/+	–/–	Expected ratio	X^2	*P* value
Observed	38	18	9	6			
Expected	39.9	13.3	13.3	4.4	9:3:3:1	2.74	0.434

[a] RAPD marker $B459_{1600}$ detected a band present (+) in the susceptible and absent (–) in the resistant plants. $B386_{900}$ detected a band present (+) in the resistant, but absent (–) in the susceptible plants.

expected ratio of the marker phenotype should be 2:1:0:0. The observed ratio of the phenotypes in the selected resistant population was consistent with this prediction (Table 5). If the two dominant complementary genes hypothesis is correct, the resistant plants selected had the genotypes AABB, AaBB, AABb, and AaBb.

The identified RAPD markers were also used to study F_3 segregating populations derived from 16 F_2 plants resistant to *M. phaseolina* (population D). The reaction of F_3 plants to the disease and the segregation of the markers indicated the genotype of the 16 resistant F_2 plants (Table 6). It was assumed that the resistant F_2 plants selected had the genotypes predicted for the presence of two dominant complementary genes (i.e., AABB, AaBB, AABb, and AaBb). In 15 of 16 F_3 populations, the genotype of the markers correlated with the genotype (heterozygous or homozygous) for the disease resistance. One plant was found to be a recombinant for RAPD marker $B459_{1600}$ (Table 6). Each RAPD marker also segregated as a dominant marker in these F_2 and F_3 populations. The ratios were consistent with the models, except for the segregation of $B386_{900}$ in population D that gave a *P* value of 0.02 to 0.05 (Table 7). The goodness-of-fit test for both markers, taking into account the genotypes identified in the F_2 plants, also determined that these two markers did not differ significantly from the expected ratio for unlinked loci in these F_2 and F_3 populations (Table 8), and possibly could be segregating independently.

TABLE 4. Segregation ratios of two RAPD markers in 68 F_2 plants that survived *Macrophomina phaseolina* inoculation from a group of 181 selected F_2 plants (population C-1) obtained from a cross between BAT-477 and A-70 that are resistant and susceptible, respectively, to *M. phaseolina*[a]

	Band		Expected ratio		
Marker	Present (+)	Absent (–)	+:–	X^2	*P* value
$B459_{1600}$					
Observed	38	30			
Expected	44	24	2:1	2.31	0.127
Expected	51	17	3:1	13.25	<0.001
$B386_{900}$					
Observed	63	5			
Expected	68	0	all present	0.36	0.544
Expected	51	17	3:1	11.29	<0.001

[a] RAPD marker $B459_{1600}$ detected a band present (+) in the susceptible and absent (–) in the resistant plants. $B386_{900}$ detected a band present (+) in the resistant, but absent (–) in the susceptible plants.

DISCUSSION

The appearance of a high fraction of the F_2 plants possessing the parental phenotypes for response to *M. phaseolina* suggested that relatively few loci were involved in the determination of the resistance phenotype. Results of this study indicated that resistance to *M. phaseolina* was controlled by two dominant complementary genes (*Mp-1* and *Mp-2*). The segregation ratio between resistant and susceptible plants in the different F_2 segregating populations studied was nearly always compatible with the expected ratio for the presence of two dominant complementary genes. The segregation of marker $B386_{900}$ in an F_3 selected resistant population (population D) was slightly distorted from the expected ratio. However, when the joint analysis was performed for both markers, the observed ratio fit the hypothesis for two unlinked loci. The involvement of two dominant complementary genes in the resistance of bean lines used in this study to *M. phaseolina* indicated that the resistance had a relatively simple genetic basis and the transfer or incorporation of these resistance genes into commercial or promising bean cultivars could be accomplish using traditional breeding methods.

The reliability of the inoculation procedure to test the reaction of beans to *M. phaseolina* played an important role in this study. The percentage of susceptible plants escaping detection was reduced by the modification made in the method developed by Abawi and Pastor-Corrales (1,18). Placing the bean seeds between two layers of soil infested with sclerotia of *M. phaseolina* increased the probability of the pathogen to infect the bean seeds or seedlings. Keeping the inoculated plants in a growth chamber for 7 days at a constant temperature of 25°C and alternating periods of 12 h of light and dark also helped to reduce the adverse environmental effects associated with the development of charcoal rot caused by *M. phaseolina* (18, G. Olaya, *unpublished data*).

The separation of resistant and susceptible categories were defined on a subjective decision. The disease severity score of 1 to 3 (resistant reaction) referred to development of restricted lesions on cotyledons and limited movement of the pathogen to stem tissues. Disease severity scores of 4 and greater indicated that the pathogen had the capability to infect the stems and advance rapidly. Because relatively few individuals were rated from 3 through 6, the precise definition of resistant and susceptible categories was not critical to the analysis. As long as categories 1 and 2 were defined as resistant and 7 to 9 were defined as susceptible, the other categories could be placed in either the resistant or susceptible group or ignored without generating a ratio that rejects the two dominant complementary genes hypothesis or making the ratio consistent with a different genetic model (e.g., 3:1, 13:3, 15:1).

Only two primers out of about 600 screened were found to display linkage to *M. phaseolina* resistance genes. Gu (7) tested about 300 primers to identify RAPD markers linked to each of the two genes that control photoperiod in beans. Miklas et al. (15) examined 167 10-mer primers to detect one RAPD linked to the *Up2* gene, a dominant resistance gene to the bean rust pathogen. Two RAPD markers from 306 primers screened were linked to a gene block that confers rust resistance in beans (8).

In this study, DNA from six plants was used to generate each of the resistant and susceptible bulks. Besides $B459_{1600}$ and $B386_{900}$, only three other RAPDs were identified in the bulk segregant analysis as possibly being linked to disease resistance. These three RAPDs proved not to be linked when tested further, but the incidence of "false positives" can be considered low. It appeared that a bulk of six individuals was sufficient in beans to identify RAPD markers linked to a trait. According to Michelmore et al. (12), when small bulks are utilized the frequency of false positives increases. Giovannoni et al. (6) also determined the negative effect of decreasing the number of samples per bulk and recommended that the pools should contain more than five individuals.

The identification of genetic markers linked to genes in beans conferring resistance to *M. phaseolina* confirmed the results of the 9:7 segregation ratio obtained in the screening of the F_2 popu-

TABLE 5. Segregation ratios of two RAPD markers in 68 F_2 plants that survived *Macrophomina phaseolina* inoculation from a group of 110 selected F_2 plants (population C-1) obtained from a cross between BAT-477 and A-70 that are resistant and susceptible, respectively, to *M. phaseolina*[a]

	Band present (+)/Band absent (–)						
	+/+	+/–	–/+	–/–	Expected ratio	X^2	*P* value
Observed	35	28	3	2			
Expected	44	24	0	0	2:1:0:0[b]	2.506	0.473

[a] RAPD marker $B459_{1600}$ detected a band present (+) in the susceptible and absent (–) in the resistant plants. $B386_{900}$ detected a band present (+) in the resistant, but absent (–) in the susceptible plants.
[b] The –/+ and –/– combination of bands were not expected.

TABLE 6. Segregation of two RAPD markers in F_3 populations derived from 16 F_2 resistant plants from the cross between the bean lines BAT-477 (resistant) and A-70 (susceptible) that survived *Macrophomina phaseolina* inoculation[a]

		$B386_{900}/B459_{1600}$ band combination				Expected band	
F_2 plant ID	Phenotype F_3[b]	+/+	+/–	–/+	–/–	combinations	Suggested genotype
AB-1	Rr[c]	0	10	0	0	all	AA Bb[d]
AB-13	RR	0	10	0	0	+/–	AA BB
AB-21	Rr	0	9	0	1	all	Aa BB
AB-30	Rr	5	2	2	1	all	Aa Bb
AB-52	Rr	7	3	0	0	all	AA Bb
AB-54	Rr	3	3	4	0	all	Aa Bb
AB-59	Rr	5	3	1	1	all	Aa Bb
AB-60	Rr	5	2	2	1	all	Aa Bb
AB-62	Rr	6	2	2	0	all	Aa Bb
AB-80	Rr	6	4	0	0	all	AA Bb
AB-83	RR	7	3	0	0	+/–	AA Bb
AB-90	Rr	6	1	3	0	all	Aa Bb
AB-101	Rr	0	9	0	1	all	Aa BB
AB-105	Rr	4	3	1	2	all	Aa Bb
AB-107	Rr	3	7	0	0	all	AA Bb
AB-116	Rr	7	3	0	0	all	AA Bb

[a] Segregation of the two markers was determined in 10 F_3 plants. RAPD marker $B459_{1600}$ detected a band present (+) in the susceptible and absent (–) in the resistant plants. $B386_{900}$ detected a band present (+) in the resistant, but absent (–) in the susceptible plants.
[b] Phenotype was determined by inoculating 10 different F_3 plants with *M. phaseolina*.
[c] RR = resistant phenotype not segregating, Rr = resistant phenotype segregating, and rr = susceptible phenotype not segregating.
[d] It seems that the plant AB-1 is a recombinant for RAPD marker $B459_{1600}$.

TABLE 7. Segregation ratios of two RAPD markers in 160 F_3 plants derived from 16 F_2 resistant plants from a cross between BAT-477 and A-70 that are resistant and susceptible, respectively, to *Macrophomina phaseolina*[a]

	Band		Expected ratio		
Marker	Present (+)	Absent (–)	+:–	X^2	*P* value
$B459_{1600}$					
F_2 - observed	12	4			
F_2 - expected	10.66	5.33	2:1	0.50	0.479
F_3 - observed	79	81			
F_3 - expected	87.6	72.4	23:19	1.86	0.171
$B386_{900}$					
F_2 - observed	16	...			
F_2 - expected	16	...	all present	...	0.999
F_3 - observed	138	22			
F_3 - expected	126	34	33:9	5.34	0.021

[a] RAPD marker $B459_{1600}$ detected a band present (+) in the susceptible and absent (–) in the resistant plants. $B386_{900}$ detected a band present (+) in the resistant, but absent (–) in the susceptible plants.

lation, indicating that two dominant complementary genes control the resistance. RAPD markers identified for *M. phaseolina* resistance genes were also observed to segregate at near 3:1 Mendelian ratios, consistent with segregation for dominant alleles at a single loci. The segregation of both markers did not differ significantly from a ratio of 9:3:3:1 expected for two unlinked loci. These RAPD markers also segregated at near the expected ratios in F_2 resistant plants that survived the inoculation with the pathogen and in F_3 populations obtained from F_2 resistant plants, which were expected to have genotype for two dominant complementary genes (i.e., AABB, AaBB, AABb, and AaBb). Weeden et al. (26) have found in segregation analysis that nearly all RAPD markers scored in segregating populations of apple and pea reflected true genetic variation that could be placed onto the respective linkage maps. They also found that there were mistakes in scoring probably caused by contamination of template DNA or faintness of the amplified product.

The two genes system controlling the resistance to *M. phaseolina* in BAT-477 complicates the estimation of the map distance. It is possible to approximate the distance between the marker $B386_{900}$ and the adjacent resistance gene using data from population C-1 (resistant plants that survived *M. phaseolina* inoculation). In this population, plants lacking the marker were presumed recombinants and the recombination frequency between the marker and the resistance gene was about 7% (five recombinants out of 68 plants). For RAPD marker $B459_{1600}$, which is linked in repulsion to the other resistance gene, the F_2 data did not give a very precise estimate of linkage intensity. However, the F_3 data (population D) could be used to obtain genotypes for the 16 F_2 parents that generated these families. Only one recombinant genotype out of the 16 F_2 plants was observed, indicating a recombination frequency between $B459_{1600}$ and the resistance gene of about 6%. To determine more precisely how tightly the linkage is between *M. phaseolina* resistance genes and the DNA markers identified, we need to identify plants homozygous for one of the genes and heterozygous for the other (e.g., AABb, AaBB), self or backcross these plants, score the backcross or F_2 population for segregation of the appropriate marker, and determine the resistance genotype of each F_2 or backcross plant by analysis of the F_3 population.

It was concluded that resistance to *M. phaseolina* in beans was conferred by two dominant complementary genes (designated as *Mp-1* and *Mp-2*). Both genes must be present, although not necessarily homozygous, to produce the resistant genotype. The

TABLE 8. Goodness-of-fit for a 17:16:6:3 segregation ratio of the two RAPD markers linked to *Macrophomina phaseolina* resistance genes in 160 F_3 plants derived from 16 F_2 resistant plants from a cross between bean lines BAT-477 (resistant) and A-70 (susceptible)[a]

	Band present (+)/Band absent (–)						
	+/+	+/–	–/+	–/–	Expected ratio	X^2	*P* value
F_2 - observed	12	4					
F_2 - expected	10.66	5.33	...	...	2:1	0.50	0.479
F_3 - observed	64	74	15	7			
F_3 - expected	65	61	23	11	17:16:6:3	6.81	0.072

[a] RAPD marker $B459_{1600}$ detected a band present (+) in the susceptible and absent (–) in the resistant plants. $B386_{900}$ detected a band present (+) in the resistant, but absent (–) in the susceptible plants.

RAPD markers $B459_{1600}$ and $B386_{900}$ should be very useful in following the inheritance of these genes and selecting resistant lines, at least when using BAT-477 and related germ plasm. We have not tested other sources of resistance to *M. phaseolina* and can not be sure that these markers will be present or useful in unrelated germ plasm. There is clearly some recombination between the markers and the respective linked genes, and the linkage of $B459_{1600}$ to the recessive (susceptible) allele reduces the utility of this marker. The DNA fragments are being cloned and sequenced to develop more convenient markers for the two genes (7,20).

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