A Single Dominant Gene in Common Bean Conferring Resistance to Three Root-Knot Nematode Species

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ABSTRACT

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Resistance to root-knot nematodes, *Meloidogyne javanica*, *M. incognita* race 1, and *M. arenaria* has been identified in common bean landraces G2618 and G1805 and in the breeding lines A445, A315, and others derived from them. The mode of inheritance of the resistance gene from the two sources was studied. The data obtained showed that the resistance from the two sources was conditioned by a single dominant gene. No segregation was observed in the F_2 populations of the crosses between resistant parents A445 × A315 and G2618 × G1805, indicating that resistance from the two sources was allelic. In the split-rooted F_2 plants of the cross A445 × cultivar Kentucky Wonder (KW) (susceptible parent) where each plant was inoculated with a different nematode isolate on each half of the root system, all the plants had the same reaction on both halves of the root system in all nematode combinations. Plants of 14 F_3 families of the cross A445 × KW showed that all families reacted the same way to *M. javanica*, *M. incognita* race 1, and *M. arenaria* race 1. These data were consistent with the hypothesis that the same gene conferred resistance to the three root-knot nematode species. We propose the symbol *Me1* for this gene.

Additional keywords: Phaseolus vulgaris.

Common bean, *Phaseolus vulgaris* L., is a highly susceptible host of root-knot nematodes (*Meloidogyne* spp.) and significant yield losses can occur if common beans are grown in nematode-infested soil (3). Use of resistant cultivars is the least expensive and most environmentally safe way to manage root-knot nematode problems in common bean and other crops. Common bean lines Alabama no. 1, PI 165426, and PI 165435 were identified as sources of resistance to *M. incognita* (Kofoid and White) Chitwood (1). Recently, this resistance was shown to be effective only against *M. incognita* races 2, 3, and 4 and not to race 1 (6). Landraces G1805 and G2618 of common bean and breeding lines derived from them have been identified as good sources of resistance to *M. incognita* race 1, *M. javanica* (Treub) Chitwood, and *M. arenaria* (Neal) Chitwood races 1 and 2 (6). The bean lines derived from the two sources had similar reaction to different isolates of *Meloidogyne* spp. However, reactions were different from those of Alabama no. 1 and PI 165426. This suggested that the resistance derived from G2618 and G1805 may be under the same genetic control, but different than that in Alabama no. 1, PI 165426, and PI 165435 (6). Furthermore, the resistance to root-knot nematodes in G2618, G1805, and lines derived from them was found to be heat stable, remaining effective at 30 C, whereas resistance in Alabama no. 1 and PI 165435 was lost at 30 C (5).

Understanding the mode of inheritance of resistance to root-knot nematodes is important in designing efficient breeding programs to incorporate resistance into desired bean cultivars. Therefore, the genetics of resistance to root-knot nematodes derived from landraces G2618 and G1805 was examined.

The objectives of this study were to 1) determine the mode of inheritance of resistance to *M. javanica*, *M. incognita* race 1, and *M. arenaria* race 1 derived from the landraces G1805 and G2618; 2) determine whether or not the resistance gene(s) from these two sources is the same; and 3) determine whether resistance to the three root-knot species is conferred by the same gene(s).

MATERIALS AND METHODS

Nematodes were multiplied on tomato (*Lycopersicon esculentum* Mill.) cultivar Tropic in the greenhouse. *M. incognita* race 1 (NCSU #54), *M. javanica* (NCSU-Mj), and *M. arenaria* race 1 (NCSU #351) obtained from North Carolina State University were used in this study. Inoculum consisting of second-stage juveniles (J2) was prepared by extracting eggs from tomato roots with NaOCl (2) and incubating the eggs in water at 25 C.

Two resistant bean lines derived from two different sources were used to determine the genetics of resistance to *M. javanica*, *M. incognita* race 1, and *M. arenaria*. Bean lines A445 and A315 were derived from the landraces G2618 and G1805, respectively, which were collected originally from Mexico (6). These landraces have been maintained and used in the common bean breeding program at Centro Internacional de Agricultura Tropical (CIAT) in Cali, Colombia. Reciprocal crosses were made between each resistant bean line and susceptible cultivar Kentucky Wonder (KW). The resulting F_1 plants were screened for resistance to *M. javanica*, *M. incognita* race 1, and *M. arenaria* race 1.

Screening plants for nematode reproduction. Surface-sterilized bean seeds were germinated in petri dishes and grown singly in seedling growth pouches (4). Seven- to ten-day-old seedlings were inoculated with 1,000 J2 of the appropriate nematode. The plants were grown in a growth chamber where temperature was maintained at 26 C and photoperiod was 16 hr. F_1 plants, and resistant and susceptible parents, were tested for resistance to the appropriate nematode. Eggs were stained with 50 mg/L of erioglaucine dye (4), and 28 days after inoculation the egg masses on plant roots were counted.

After evaluation the plants were transferred into 15-cm-diameter fiber pots filled with UC-mix soil and grown to maturity in the greenhouse to obtain F_2 seeds and also for backcrossing to the resistant and susceptible parents. The F_2 plants from crosses A445 × KW and A315 × KW, and the resistant and susceptible parents, were screened for resistance to *M. incognita* race 1, *M. javanica*, and *M. arenaria* race 1 as described. To confirm genetic ratios from the F_2 generation the backcross populations of the two crosses were screened also for resistance to *M. javanica*.

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Crosses were made also between plants of resistant lines in the combinations, A445 × A315 and G2618 × G1805. These crosses were used to determine whether resistance derived from the two landraces, G2618 and G1805, was conditioned by the same or different gene loci. F_1 and F_2 populations of the two crosses were screened for resistance to *M. javanica*.

Split-root systems of F_2 plants of the cross A445 × KW were inoculated with three isolates of root-knot nematodes to determine whether or not the same gene confers resistance to the three nematode isolates. Two seedling growth pouches were stapled together and a rectangular slot cut in the two adjoining inside surfaces of the growth pouches. One germinating bean seedling was placed in the slot and roots of each seedling were allowed to grow into both growth pouches. Each side was inoculated with 1,000 J2 of a different nematode isolate from the following combinations, *M. javanica*/*M. arenaria* race 1, *M. javanica*/*M. incognita* race 1, and *M. arenaria* race 1/*M. incognita* race 1. Plants were maintained and evaluated as described before.

After evaluation, selected resistant and susceptible F_2 plants of the cross A445 × KW were transferred to 15-cm-diameter pots and grown to maturity to obtain F_3 seeds. In order to obtain corroborative data to determine whether or not the same gene confers resistance to the three nematode species, families of F_3 seedlings obtained from 14 F_2 plants were screened for resistance to the three root-knot nematode species. All seedlings obtained from each F_2 plant constituted a family. Approximately 30 F_3 plants from each family were screened, 10 plants to each nematode, for resistance to *M. incognita* race 1, *M. javanica*, and *M. arenaria* race 1.

Designation of resistance. Plants were classified as resistant if they supported 12 or fewer egg masses per root system and susceptible if they supported more than 12 egg masses. Most of the resistant plants had zero egg masses, but some had egg mass numbers ranging from 1 to 12. Egg mass numbers on resistant parents, and the F_1 plants did not exceed 12 in all experiments. Greater variability in egg mass numbers was observed in susceptible plants, which ranged from 15 to more than 100.

RESULTS

The F_1 plants from reciprocal crosses between the resistant lines A445, A315, and susceptible cultivar Kentucky Wonder (KW) supported low average numbers of egg masses (less than 5) of *M. javanica*, *M. incognita* race 1, and *M. arenaria* race 1 (Table 1). Some F_2 plants from the two crosses A445 × KW and A315 × KW supported very low egg mass numbers (fewer than three) of the three nematode species, while other F_2 plants from the two crosses supported a high average of egg mass numbers (33–162) (Table 1). Variability in egg mass numbers was observed in all tests. The variability could be attributed mainly to differences in size of root systems. An example of the distribution of plants with different numbers of egg masses is given in Table 2 for plants of the parental, F_1, and F_2 generations of the cross A315 × KW. Reaction to nematodes of the progeny of plants designated as resistant or susceptible was as expected in all tests, indicating that the criterion of $\leq$ or $>$ 12 egg masses for designating resistant and susceptible classes, respectively, was valid.

The F_2 plants from both crosses (A445 × KW and A315 × KW) segregated 3 resistant:1 susceptible to the nematode species. The chi-square and *P* values for each test are given in Table 3. To verify the F_2 ratios, backcross populations from the two crosses were screened for resistance to *M. javanica*. The plants backcrossed to susceptible KW segregated 1 resistant:1 susceptible and those backcrossed to the resistant parents were all resistant (Table 4).

Crosses between resistant lines were made to determine the allelic relationship between the dominant gene in lines A445 and A315. Resistance in these two lines was derived from the landraces G2618 and G1805, respectively, which were crossed to obtain corroborative data. The F_1 and F_2 populations from the crosses supported very low nematode reproduction. Thus no segregation was observed in F_2 populations of the crosses A445 × A315 and G2618 × G1805 (Table 5).

All the F_2 plants inoculated with a combination of two nematode isolates showed the same reaction on both halves of the roots (Table 6). Thus, each plant that had a resistant reaction to one nematode isolate on one half of the root showed a resistant reaction to a different nematode isolate on the other half of the root system. The same was true with the susceptible reaction. Segrega-

TABLE 1. Reproduction of *Meloidogyne javanica*, *M. incognita* race 1, and *M. arenaria* race 1 on parental lines and different generations of the crosses A445 × Kentucky Wonder (KW), A315 × KW, (A445 × KW) × KW, (A445 × KW) × A445, (A315 × KW) × KW, (A315 × KW) × A315, A445 × A315, and G2618 × G1805 of common bean

Generation	*M. javanica*	*M. incognita*	*M. arenaria*
KW[a]	117.0 ± 95.0[b]	118.0 ± 66.0	119.0 ± 50.0
A445	0.3 ± 0.8	0.0	0.1 ± 0.3
A315	0.5 ± 0.9	0.0	0.1 ± 0.4
A445 × KW (F_1)	2.7 ± 4.0	4.3 ± 5.0	0.0
A445 × KW (F_2)-S[c]	33.0 ± 19.0	140.0 ± 83.0	162.0 ± 89.0
A445 × KW (F_2)-R[d]	1.6 ± 2.5	2.3 ± 4.0	1.2 ± 2.2
A315 × KW (F_1)	2.3 ± 3.0	0.5 ± 0.7	0.5 ± 0.6
A315 × KW (F_2)-S	106.0 ± 66.0	84.0 ± 63.0	103.0 ± 60.0
A315 × KW (F_2)-R	0.9 ± 7.0	0.3 ± 1.0	0.3 ± 0.9
(A445 × KW) × A445	0.05 ± 0.2	...[e]	...
(A445 × KW) × KW-S	66.0 ± 30.0	...	...
(A445 × KW) × KW-R	0.5 ± 1.8	...	...
(A315 × KW) × A315	0.0	...	...
(A315 × KW) × KW-S	65.0 ± 44.0	...	...
(A315 × KW) × KW-R	0.5 ± 1.0	...	...
A445 × A315	0.35 ± 1.2	...	...
G2618 × G1805	0.6 ± 1.8	...	...

[a] KW, susceptible parent; A445 and A315 are resistant parents. Values for parental lines are combined for all tests.
[b] Values are average number of egg masses. Reproduction is measured by egg mass number per root system ± standard deviation.
[c] Susceptible plants of the segregating population.
[d] Resistant plants of the segregating population.
[e] Not tested.

TABLE 2. Distribution of egg mass numbers in parental, F_1 and F_2 plants of the cross A315 × Kentucky Wonder (KW) inoculated with *Meloidogyne javanica*

	Number of plants				
Egg mass number	KW	A315	A315 × KW (F_1)	A315 × KW (F_2)	
0	0	7	4	132	Resistant 163
1–5	0	3	7	29	
6–10	0	0	2	1	
11–12	0	0	0	1	
13–20	0	0	0	0	Susceptible 50
21–30	0	0	0	6	
31–50	1	0	0	5	
51–100	9	0	0	15	
>100	2	0	0	24	
Total	12	10	13	213	

tion for resistance to the nematode isolates fitted the ratio 3 resistant:1 susceptible (Table 6).

From the cross A445 × KW, nine F_2 plants resistant to *M. javanica* and five F_2 plants that were susceptible were allowed to self to obtain F_3 populations. An F_3 family derived from a resistant F_2 plant was designated as segregating if one or more plants were susceptible, otherwise the family was designated as all resistant. Plants of families from susceptible F_2 plants were all susceptible to each nematode isolate. Of the nine families derived from resistant F_2, six did not segregate (all resistant to each nematode isolate) and three segregated for resistance. This gave a ratio of 2 all resistant:1 segregating families (Table 7). A ratio of 3 resistant:1 susceptible to *M. javanica* ($\chi^2 = 0.88$, $P = 0.50$–0.75), to *M. incognita* race 1 ($\chi^2 = 0.36$, $P = 0.75$–0.90), and to *M. arenaria* race 1 ($\chi^2 = 0.60$, $P = 0.50$–0.75) was obtained for plants from segregating families.

DISCUSSION

Our results show that resistance to *M. javanica*, *M. incognita* race 1, and *M. arenaria* race 1 in common bean lines A445 and A315 is controlled by a single dominant gene. These bean lines were derived from landraces G2618 and G1805, respectively (6). It has been proposed that resistance from the two sources is under the same genetic control (6). To test this hypothesis F_1 and F_2 populations of the crosses between resistant parents A445 × A315 and G2618 × G1805 were screened for resistance to *M. javanica*. Lack of segregation in F_2 of these crosses indicated that resistance from the two sources was conditioned by the same gene locus.

Under a single dominant gene hypothesis, the resistant F_2 plants of the cross A445 × KW should be a mixture of heterozygotes and homozygotes; therefore, some F_3 families should segregate and others should not. The families should segregate 2 all resistant:1 segregating, and plants in segregating families should segregate 3 resistant:1 susceptible. Our observed data fitted closely these expected segregation ratios in both cases, supporting a single dominant gene mode of inheritance for root-knot nematode resistance.

Under the hypothesis that resistance to *M. javanica*, *M. incognita* race 1, and *M. arenaria* race 1 was conditioned by the same gene, the F_3 plants from the same family would have the same reaction to each of the nematode species. Thus, if a family segregated for resistance to one nematode species, it should segregate for resistance to the other two species and vice versa. Nonsegregating resistant families should be all resistant to the three nematode species, and the susceptible families should breed true for susceptibility to the three nematode species. This was found to be the case, with six nonsegregating resistant families, plants from three resistant families segregating 3 resistant:1 susceptible to each nematode species, and five nonsegregating susceptible families.

The split-root experiment made it possible to examine the segregation for resistance to two nematode isolates simultaneously on the same plant without cross contamination. It was assumed that no interactive effect occurs due to split-root technique. All plants segregating for resistance or susceptibility to one nematode isolate segregated for resistance or susceptiblity to the other isolate. If resistance to the three nematode isolates was controlled by different genes, it would be expected that in some plants one side of the root system would have had a different reaction from

TABLE 3. Reaction of F_1 and F_2 populations of the common bean crosses, A445 × Kentucky Wonder (KW) and A315 × KW to *Meloidogyne javanica* (Mj), *M. incognita* race 1 (Mi1), and *M. arenaria* race 1 (Ma1)

Cross	Nematode isolate	Obs. freq. R[a]	Obs. freq. S[b]	Expected ratio	Chi-square	*P* value
A445 × KW(F_1)	Mj	100	0	All R	···	···
A445 × KW(F_2)	Mj	164	53	3:1	0.04	0.8–0.9
A315 × KW(F_1)	Mj	13	0	All R	···	···
A315 × KW(F_2)	Mj	163	50	3:1	0.26	0.5–0.7
A445 × KW(F_1)	Mi1	4	0	All R	···	···
A445 × KW(F_2)	Mi1	124	43	3:1	0.05	0.8–0.9
A315 × KW(F_1)	Mi1	4	0	All R	···	···
A315 × KW(F_2)	Mi1	84	27	3:1	0.03	0.8–0.9
A445 × KW(F_1)	Ma1	4	0	All R	···	···
A445 × KW(F_2)	Ma1	132	44	3:1	0.76	0.3–0.5
A315 × KW(F_2)	Ma1	4	0	All R	···	···
A315 × KW(F_2)	Ma1	85	27	3:1	0.05	0.8–0.9

[a]R indicates resistant reaction: 12 or fewer egg masses per root system.
[b]S indicates susceptible reaction: more than 12 egg masses per root system.

TABLE 4. Reaction of backcross populations of the crosses A445 × Kentucky Wonder (KW) and A315 × KW of common bean to *Meloidogyne javanica*

Cross	Obs. freq. R[a]	Obs. freq. S[b]	Expected ratio	Chi-square	*P* value
(A445 × KW) × KW	24	25	1:1	0.02	0.9
(A445 × KW) × A445	20	0	All R	···	···
(A315 × KW) × KW	14	18	1:1	0.50	0.3–0.5
(A315 × KW) × A315	10	0	All R	···	···

[a]R indicates resistant reaction: 12 or fewer egg masses per root system.
[b]S indicates susceptible reaction: more than 12 egg masses per root system.

TABLE 5. Reaction of F_1 and F_2 populations of resistant bean lines to *Meloidogyne javanica*

Cross	Observed frequency Resistant[a]	Observed frequency Susceptible[b]
Kentucky Wonder	0	9
A315	10	0
A445	10	0
G1805	12	0
G2618	9	0
A315 × A445(F_1)	6	0
G1805 × G2618(F_1)	11	0
A315 × A445(F_2)	139	0
G1805 × G2618(F_2)	134	0

[a]Resistant plants have 12 or fewer egg masses per root system.
[b]Susceptible plants have more than 12 egg masses per root system.

TABLE 6. Reaction of split-rooted F_2 plants of the cross A445 × Kentucky Wonder (KW)[a]

Cross	Mj/Ma1[b] R/R[c]	Mj/Ma1[b] S/S[c]	Mj/Ma1[b] *P* value[d]	Mj/Mi1 R/R	Mj/Mi1 S/S	Mj/Mi1 *P* value	Ma1/Mi1 R/R	Ma1/Mi1 S/S	Ma1/Mi1 *P* value
KW	0/0	2/2[e]	···	0/0	2/2	···	0/0	2/2	···
A445	2/2	0/0	···	1/1	0/0	···	2/2	0/0	···
A445 × KW	15/15	4/4	>0.90	17/17	6/6	>0.90	12/12	4/4	>0.90

[a]Each half of the root system inoculated with *Meloidogyne javanica* (Mj), *M. incognita* race 1 (Mi1) or *M. arenaria* race 1 (Ma1).
[b]One half of the root system of each plant was inoculated with *M. javanica* and the other half with *M. arenaria* race 1.
[c]R/R or S/S means resistant or susceptible reaction on both halves of the root system of a plant. No R or S reaction differed on both sides of root for any plant tested.
[d]*P* values are for 3:1 expected ratio of resistant to susceptible plants. Yates' correction factor (8) was used in the calculation of chi-square.
[e]2/2 means two plants, one side of each plant root system is susceptible to *M. javanica* and the other side is susceptible to *M. arenaria* race 1.

TABLE 7. Reaction of F_3 populations of the cross A445 × Kentucky Wonder (KW) to *Meloidogyne javanica*, *M. incognita* race 1, and *M. arenaria* race 1

Family	*M. javanica*	*M. incognita*	*M. arenaria*
Resistant[a]			
7-A445 × KW	Seg[b]	Seg	Seg
24-A445 × KW	All R[c]	All R	All R
61-A445 × KW	Seg	Seg	Seg
28-A445 × KW	All R	All R	All R
110-A445 × KW	All R	All R	All R
9-A445 × KW	All R	All R	All R
165-KW × A445	All R	All R	All R
164-KW × A445	All R	All R	All R
185-KW × A445	Seg	Seg	Seg
Susceptible[d]			
25-A445 × KW	All S[e]	All S	All S
20-A445 × KW	All S	All S	All S
144-KW × A445	All S	All S	All S
147-KW × A445	All S	All S	All S
140-KW × A445	All S	All S	All S

[a] F_3 families from resistant F_2 plants.
[b] In segregating families (seg) some plants are susceptible and others are resistant.
[c] All the plants in the family are resistant (have 12 or fewer egg masses).
[d] F_3 families from susceptible F_2 plants.
[e] All plants in the family show a susceptible reaction (have more than 12 egg masses per root system).

the other half inoculated with a different isolate. However, since this was not the case, it is apparent that these data and those from the F_3 families are consistent with the hypothesis that the same gene confers resistance to the three nematode species.

This is the first report of a single dominant gene in common bean conditioning resistance to root-knot nematode species. These data provide evidence that resistance in all the breeding lines from CIAT derived from G2618 and G1805 is controlled by the same locus. Furthermore, it is the same locus that conditions resistance to *M. javanica*, *M. incognita* race 1, and *M. arenaria*. Since this is the first dominant gene identified in common bean that confers resistance to *Meloidogyne* spp., we propose the symbol *Me1* to denote this gene.

The simple nature of inheritance of this resistance suggests that it can be incorporated relatively easily into suitable bean cultivars. Furthermore, this gene could be introduced with relative ease into cultivars with resistance to *M. incognita* races 2, 3, and 4 present in Alabama no. 1, PI 165426, and PI 165435 (1,6). However, since resistance to *M. javanica*, *M. incognita* race 1, and *M. arenaria* from the two sources (G2618 and G1805) is controlled by the same dominant gene representing a narrow genetic base, the identification of additional sources of resistance will be important because repeated use of this resistance may select resistance-breaking biotypes. This has occurred in other crops such as tomato (7).

LITERATURE CITED

1. Fassuliotis, G., Deakin, J. R., and Hoffman, J. C. 1970. Root-knot nematode resistance in snap bean: Breeding and nature of resistance. J. Am. Hortic. Sci. 95:640-645.
2. Hussey, R. S., and Barker, K. R. 1973. A comparison of methods of collecting inocula of *Meloidogyne* spp. including a new technique. Plant Dis. Rep. 57:1025-1028.
3. Ngundo, B. W., and Taylor, D. P. 1974. Effects of *Meloidogyne* spp. on bean yields in Kenya. Plant Dis. Rep. 58:1020-1023.
4. Omwega, C. O., Thomason, I. J., and Roberts, P. A. 1988. A nondestructive technique for screening bean germ plasm for resistance to *Meloidogyne incognita*. Plant Dis. 72:970-972.
5. Omwega, C. O., Thomason, I. J., and Roberts, P. A. 1990. Effect of temperature on expression of resistance to *Meloidogyne* spp. in common bean (*Phaseolus vulgaris*). J. Nematol. (in press).
6. Omwega, C. O., Thomason, I. J., Roberts, P. A., and Waines, J. G. 1989. Identification of new sources of resistance to root-knot nematodes in *Phaseolus*. Crop Sci. 29:1463-1468.
7. Roberts, P. A., and Thomason, I. J. 1989. A review of variability in four *Meloidogyne* spp. as measured by reproduction on several hosts including *Lycopersicon*. Agric. Zool. Rev. 3:225-252.
8. Stansfield, W. D. 1983. Theory and Problems of Genetics. McGraw-Hill Book Co., Inc., New York. 392 pp.