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Evidence for simply inherited dominant resistance to *Meloidogyne javanica* in carrot

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Abstract Root-knot nematodes (*Meloidogyne* spp.) are serious pests of carrot (*Daucus carota* L.) worldwide. While soil treatment with nematicides is the primary means for managing nematodes in carrot, there is a need to identify and introduce host plant resistance for crop improvement. This study was conducted to determine the inheritance of resistance to root-galling and reproduction by *M. javanica* (Treub) Chitwood in a selection (BR-1252) of carrot variety Brasilia. F_2, F_3, F_4, and BC_1 progenies from the cross BR-1252×B6274 (a susceptible inbred line) were screened in pot tests for reaction to *M. javanica*. The observed reactions based on galling and egg production on fibrous roots gave segregation patterns in all tests that were consistent with relatively simply inherited dominant resistance. Field testing in progress indicates that this resistance is very effective against both *M. javanica* and *M. incognita*. A single gene model fits the observed data acceptably well in F_3 generations. However, the range of 3% to 51% susceptible plants in segregating F_3 families and 1% to 47% in segregating F_4 families is much wider than the 25% expected with a single-gene model, and linked duplicate factors in the coupling phase could also explain the observed segregation patterns. The variation in percentage susceptibility among these families did not clearly cluster into three expected categories (25% S, 20.25% S, and 0.25% S for a 10-cM linkage distance, or 25% S, 16% S and 1% S for 20 cM), but it did tend to occur over the same range. Thus a 10-cM to 20-cM-linked duplicate factor model cannot be dismissed at this time. Egg production data in the F_2, F_3, and F_4 families provided evidence for slightly lower resistance expression in the heterozygous condition. Thus, while overall expressed in a dominant fashion, the resistance does exhibit some allelic dosage response.

Key words Carrot · *Daucus carota* L. · Disease resistance · *Meloidogyne javanica* · Root knot nematodes

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Introduction

The attack of carrots (*Daucus carota* L.) by root-knot nematodes severely limits marketable yields in much of the world. *Meloidogyne* species, including *M. javanica* (Treub) Chitwood, *M. incognita* (Kofoid and White) Chitwood, and *M. arenaria* (Neal) Chitwood, are usually responsible in warmer production areas, while *M. hapla* Chitwood is a significant problem in temperate regions (Huang et al. 1986; Roberts et al. 1988; Vrain 1982). The 'cosmetic injury' caused by the forking and galling symptoms on the marketable taproot is responsible for the significant yield losses that occur on carrot (Roberts 1987). Currently, preplant fumigation of soil with such nematicides as 1,3-dichloropropene, metam-sodium, or methyl bromide provide the standard management strategy for root-knot nematode in carrot (Roberts et al. 1988). Some opportunities for nematode management are also available through the cultural tactics of manipulating the planting and harvesting dates of carrots to avoid periods of the year when the nematode is most active and infective in soil (Roberts 1987). There have been reports of resistance to *M. javanica* from several carrot varieties including 'Brasilia' (Charchar and Viera 1994; Huang 1986; Huang et al. 1986). However, commercial carrot cultivars worldwide are typically root-knot nematode susceptible.

Due to the high sensitivity of the carrot taproot to the damaging effects of nematode infection and concerns about the future availability of nematicides, the development of root-knot nematode-resistant carrot cultivars is

highly desirable. In an evaluation of a broad-based collection of carrot germplasm we also found strong resistance, approaching immunity in some selections of 'Brasilia'. This report documents the genetic nature of *M. javanica* resistance derived from 'Brasilia'. Evidence for simply inherited dominant resistance is presented.

Materials and methods

Nematode resistance screening

Root-knot nematodes (*M. javanica* isolate Project 811 from California) used for evaluation of carrot resistance were maintained and multiplied on tomato cv 'Tropic'. Tomato seedlings were grown in a greenhouse in 18-cm pulp pots filled with blow sand and inoculated with approximately 50000 eggs 2 weeks after transplanting. After 50–55 days, tomato plants were removed from the pots, the roots rinsed and cut into 2- to 4-cm segments, macerated in NaOCl, and nematode eggs collected with sieves (Hussey and Barker 1973).

Carrot populations were evaluated for nematode resistance under controlled conditions in a greenhouse. Individual carrot seeds were planted into 10-cm-diameter plastic pots filled with fine blow sand. Where direct-planted seed failed to germinate, additional carrot seeds were germinated on filter paper in petri dishes and transferred to those pots without plants. Each pot was fertilized with daily application of full-strength Hoagland's solution (Hoagland and Arnon 1950) for about the first 7 days after plant emergence. For the remainder of the test, plants were fertilized by a surface application of 6 g of a 17–6–10 controlled release fertilizer (Scotts-Sierra Horticultural Products Co). Seedlings with three true leaves (about 1 month after planting) were inoculated through a syringe with approximately 30000 eggs of *M. javanica* in 10 ml water at two places in the pot (5 ml each). The 7-cm-long syringe needle had three holes drilled at 1.5-cm intervals to facilitate uniform inoculation with depth. Pots were drip-irrigated as needed to maintain optimal growth. Air temperatures in the greenhouse were maintained between 27° and 35° C during the day and at 24°±1° C at night. Soil temperatures were in the range of 24°–27° C during the experiments.

Carrots were evaluated for a resistance reaction approximately 60 days after inoculation. A 0–4 gall rating scale was used to categorize the visible root-knot damage on carrot fibrous roots. Effects of root-knot nematode on tap roots of plants grown in pots are more difficult to assess than symptoms on fibrous roots. This scale was modified to include a 'trace' category, and carrots were indexed for galling as follows: 0=no galls, trace=very few difficult to find galls, 1=1–25%, 2=26–50%, 3=51–75%, and 4=76–100% of fibrous roots galled. Because some carrots had an intermediate rating (i.e., 1–2, 2–3, or 3–4), all carrot scores were doubled to give whole numbers (e.g., trace was converted to 1, 1 converted to 2, 1–2 converted to 3, and so on). The resulting 0–8 scale was used for hypothesis testing. Plants of cv 'Imperator 58' were evaluated for comparison as a susceptible check.

Carrot resistance was also evaluated by assessing the numbers of nematode eggs per root system and per gram of fresh fibrous root. Fibrous roots were carefully removed from the tap root and eggs extracted from the fibrous roots with NaOCl (Hussey and Barker 1973) and counted. A $\log_{10}$ (n + 1) transformation was applied to the egg data prior to statistical analyses. The transformation was needed to equalize variances among treatment means because of the positive correlation between means and variances. Reproduction was expressed as $\log_{10}$ mean eggs per root system and $\log_{10}$ mean eggs per gram of root. Egg data were analyzed with the SAS ANOVA program (SAS 1985).

Plant populations

Preliminary evaluation of 50 hybrid and open-pollinated carrot cultivars and USDA inbreds indicated little evidence for resistance to *M. javanica*, but some individual plants of 'Brasilia' were resistant. Seven F_2 populations derived from crosses with 'Brasilia' were evaluated and one population of 'Brasilia–1252' ('BR-1252')×B6274 with a high incidence of resistance (derived from a single F_1 plant) was selected for detailed evaluation. B6274 is a USDA inbred susceptible to *M. javanica* (gall rating ≥3). Of 470 F_2 individuals evaluated, 104 covering the range of gall ratings were self-pollinated for evaluation of F_3 segregation and 33 selected resistant F_3s were, in turn, self-pollinated to evaluate F_4 progeny and in some cases backcrossed to the male-sterile version of the susceptible parent, B6274, for further progeny testing. Estimates of minimum family sizes to differentiate between segregation ratios and to identify a recessive segregant were calculated from formulae for examples C and A, respectively, of Hanson (1959).

Results and discussion

A high incidence of resistant progeny was observed in the F_2 family of BR-1252×B6274 with 294 of 470 plants demonstrating no gall production (Table 1). A bimodal distribution for gall rating (GR) was noted with separation at GR 2 or GR 3 on the 0–8 scale. Of 16 F_3 families from the more susceptible F_2 plants (GR 3–GR 8), all were uniformly susceptible with gall ratings for each family ranging between GR 7–GR 8 and GR 3–GR 8, except for 1 plant with a gall rating of 2. Progeny of more heavily galled plants within this group, i.e., GR 6 or GR 8, had a similar range of response to those progeny of less heavily galled plants, i.e., GR 3 or GR 4, suggesting that plants with a gall rating of 3 were susceptible and genetically equivalent to those with a gall rating of 8.

Of 88 F_3 families from more resistant F_2 plants ranging from GR 0 to GR 1, three categories of F_3 family distribution were observed. From resistant F_2 plants, 3 (GR 0) had susceptible (GR 3–GR 8) progeny, 30 had uniformly resistant progeny (GR 0–GR 2), and 55 had segregating progeny covering the full range of gall rating, GR 0 through GR 8.

The three resistant F_2s which generated uniformly susceptible F_3 progeny were apparently due to a misclassification of F_2 plants. For 1 of these (family no. 276) two F_4 progeny families from resistant (GR O and GR 1) and 1 from susceptible (GR 8) F_3 plants were evaluated and found to be uniformily susceptible (Table 2) to prove that this F_2 was mis-classified. This error rate of 3/88 or 3.4% reflects a high level of accuracy in the evaluation of nematode resistance. This error rate also suggests that the observed incidence of resistance in the F_2 is slightly overestimated. The fact that 1 plant with a gall rating of 2 (in the resistant range) was observed among the more than 500 F_3 progeny of susceptible F_2 plants (Table 1) also suggests a slight overestimation in the level of resistance.

Among the 30 F_3 families with uniformly resistant progenies, the infection response was within the GR 0–GR 2 range for all individuals (Table 1). Segregating F_3 and F_4 families derived from resistant F_2 and F_3 plants, and F_4 families from resistant F_3 plants, had a wide range in the incidence of susceptible plants (GR

Table 1 Variation in response to *Meloidogyne javanica* in a carrot F_2 family and F_3 progeny

F_2 generation score	Gall rating									Total	F_3 families tested with same range of gall rating	Percentage of plants with gall rating ≥3 for F_3 families
	0	1	2	3	4	5	6	7	8			
F_2	294	78	8	3	35	11	32	1	8	470		
F_3 progeny examples												
From susceptible F_2s												
4	0	0	0	0	0	0	2	1	40	43	1	100
4	0	0	0	0	0	2	16	8	14	40	2	100
4	0	0	0	0	2	1	6	0	16	25	1	100
4	0	0	0	1	4	1	9	0	9	24	1	100
5	0	0	0	0	0	0	3	7	32	42	1	100
5	0	0	0	0	3	5	5	5	19	37	2	100
5	0	0	1	0	0	1	3	6	33	44	1	98
6	0	0	0	0	0	0	2	5	33	40	2	100
6	0	0	0	0	0	3	5	4	11	23	1	100
6	0	0	0	0	4	2	3	7	21	37	1	100
6	0	0	0	1	2	3	5	2	10	23	2	100
8	0	0	0	0	0	0	0	2	20	22	1	100
											16	
From resistant F_2s (Uniformly susceptible F_3s)												
0	1	0	0	0	1	0	1	7	14	24	3	95, 96, 100
(Uniformly resistant F_3s)												
0	23	0	0	0	0	0	0	0	0	23	10	0
0	28	11	0	0	0	0	0	0	0	39	15	0
0	27	6	3	0	0	0	0	0	0	36	3	0
1	12	12	0	0	0	0	0	0	0	24	1	0
1	11	12	1	0	0	0	0	0	0	24	1	0
											30	
(Segregating F_3s)												
0	22	0	0	0	0	1	1	0	1	25	2	9, 12
0	17	0	0	1	1	1	1	0	1	22	2	23, 29
0	14	6	0	0	0	0	0	0	4	24	2	5, 17
0	7	6	0	0	0	0	0	1	3	17	4	7, 18, 24, 25
0	20	5	0	0	0	0	1	1	1	28	5	3, 4, 11, 14, 16
0	27	10	0	0	0	1	0	0	2	40	11	7, 8, 8, 14, 15, 17, 19, 22, 25, 26, 28
0	24	15	0	0	1	0	1	0	8	49	7	9, 10, 15, 19, 20, 27, 51
0	12	4	0	1	0	0	1	0	2	20	2	3, 20
0	19	2	1	0	0	0	0	1	0	23	1	4
0	11	10	1	0	0	0	1	0	2	25	1	12
0	9	2	1	0	1	2	4	1	2	22	2	23, 45
0	26	1	1	1	0	1	3	3	1	37	1	24
											40	
1	34	0	0	0	0	1	4	0	1	40	1	15
1	19	3	0	0	0	0	0	0	1	23	1	4
1	18	1	0	0	0	0	3	0	1	23	2	17, 17
1	31	1	0	0	0	1	2	2	2	39	4	8, 18, 28, 28
1	16	2	0	0	1	1	1	1	0	22	1	18
1	17	9	0	1	0	0	2	1	4	34	2	4, 24
1	13	0	1	0	0	1	2	1	1	19	1	26
1	29	9	1	0	1	1	3	1	0	45	1	13
1	2	16	1	2	0	0	1	0	2	24	2	21, 36
											15	
										Grand total: 104 families		

≥3): from 3% to 51% among F_3 families (Table 1) and from 1% to 47% among F_4 families (Table 2). Most families were in the 7%–32% range, and the percentages of susceptible plants were relatively evenly distributed throughout this range for F_3 families while F_4 families were either in the less than 1–2% range (3 families) or in the 22–47% range (11 families).

Uniformly resistant F_3 families were usually derived from F_2 plants with a gall rating of 0, whereas F_2 plants with a gall rating of 1 were more likely to generate segregating F_3 families; therefore, the F_2 rating in general was a fair predictor of F_3 family distribution (Table 1). Analysis of galling reaction in F_4 families provided a similar pattern of reaction distributions as those of the F_3

Table 2 Variation in response to *Meloidogyne javanica* in carrot F_4 and BC_1 (to susceptible parent) progenies derived from root-gall rated F_3 plants (*ns* not significant)

F_3 generation score	Progeny family	Progeny incidence		Total	Percentage S	Expected ratio	χ^2
		R=0–2	S=3–8				
(Family no.276: F_2 score=0, F_3 family 95% S)							
0	F_4	0	74	74	100	0:1	0 ns
1	F_4	0	66	66	100	0:1	0 ns
8	F_4	0	40	40	100	0:1	0 ns
(Family no. 422: F_2 score=0, F_3 family 51% S)							
0	F_4	42	0	42	0	1:0	0 ns
0	F_4	40	15	55	27	3:1	0.14 ns
	BC_1	11	16	57	59	1:1	0.92 ns
0	F_4	7	2	9	22	3:1	0.04 ns
	BC_1	37	31	68	46	1:1	0.53 ns
8	F_4	0	29	29	100	0:1	0 ns
	BC_1	0	58	58	100	0:1	0 ns
(Family no. 489: F_2 score=1, F_3 family 36% S)							
0	F_4	62	0	62	0	1:0	0 ns
0	F_4	47	0	47	0	1:0	0 ns
0	F_4	8	7	15	47	3:1	3.75 ns
0	F_4	47	14	61	23	3:1	0.13 ns
1	F_4	29	12	41	29	3:1	0.40 ns
1	F_4	72	32	104	31	3:1	1.84 ns
2	F_4	25	19	44	43	3:1	7.76**
7	F_4	0	43	43	100	0:1	0 ns
8	F_4	0	21	27	100	0:1	0 ns
(Family no. 192 A: F_2 score=0, F_3 family 18% S)							
0	F_4	162	0	162	0	1:0	0 ns
0	F_4	141	0	141	0	1:0	0 ns
0	F_4	145	1	146	<1	1:0	–
0	F_4	124	3	127	2	1:0	–
0	F_4	73	33	106	31	3:1	2.13 ns
1	F_4	51	1	52	2	1:0	–
1	F_4	122	48	170	28	3:1	0.95 ns
1	F_4	70	33	103	32	3:1	2.72 ns
1	F_4	30	21	51	41	3:1	7.12**
7	F_4	2	31	33	94	0:1	–
	BC_1	0	28	28	100	0:1	0 ns
8	F_4	0	64	64	100	0:1	0 ns
(Family no. 59: F_2 score=0, F_3 family 3% S)							
0	F_4	28	0	28	0	1:0	0 ns
0	F_4	74	0	74	0	1:0	0 ns
0	F_4	113	0	113	0	1:0	0 ns
1	F_4	92	0	92	0	1:0	0 ns
(Family no. 112: F_2 score=0, F_3 family 0% S)							
0	F_4	30	0	30	0	1:0	0 ns
	BC_1	72	0	72	0	1:0	0 ns
1	F_4	47	0	47	0	1:0	0 ns
	BC_1	19	0	19	0	1:0	0 ns

** $P \leq 0.05$

families. Although the GR of F_3 plants was not always predictive of F_4 family distribution, most F_4 families derived from F_3s with GR 0 were uniformly resistant, whereas most with GR 1 or GR 2 were segregating (Table 2).

Overall, among the resistant portion of segregating F_3 families the prevalent gall ratings were GR 0 or GR 1, whereas among the susceptible portion of these families the prevalent gall ratings were GR 6, GR 7, and GR 8. However, the range of infection response varied somewhat among segregating F_3 families. Only completely resistant plants (GR=0) were observed in the resistant grouping of some F_3 families, while others had individual segregants with gall ratings of 1 and 2 as well. Similarly, the susceptible grouping of segregating F_3 families only included plants with a gall rating of 8 in some cases while in others that grouping included plants ranging from GR 3 to GR 8. Thus the distributions of resistant and susceptible progenies in segregating F_3 and F_4 families were comparable with the distributions of progenies in uniformly resistant and uniformly susceptible families, respectively.

Nematode reproduction based on egg production on fibrous roots was compared to the GR scores of their respective F_2 and F_3 parental plants. (Tables 3 and 4). The data from each of these generations demonstrated a strong positive correlation between egg production and GR. These results provide convincing evidence for the

Table 3 Egg production of *Meloidogyne javanica* isolate Project 811 on F_2 carrots from the cross BR1252×B6274

F_2 carrot fibrous root gall rating	Number of plants tested	Mean total eggs	Mean eggs per gram of root
8	7	68619	17334
7	1	53389	17796
6	32	82844	14661
5	11	70934	11071
4	35	56068	8728
3	3	45741	5164
2	8	5854	1935
1	74	2672	804
0	290	823	255

Table 4 Egg production of *Meloidogyne javanica* isolate 'Project 811' on randomly selected F_3 and F_4 carrots from the cross BR1252×B6274

Carrot fibrous root gall rating	Number of plants tested	Mean total eggs	Mean $\log_{10}$ (total eggs+1)	Mean eggs per gram of root	Mean $\log_{10}$ eggs per gram of root +1)
F_3 plants					
8	10	249244	5.38 a	23208	4.36 a
6	10	248778	5.38 a	29345	4.46 a
1	10	27111	4.3 b	8518	3.82 b
0	10	3878	3.10 c	1357	2.85 c
lsd (P=0.05)			0.36		0.34
F_4 plants					
8	6	173333	5.21 a	24301	4.36a
6	6	105759	5.00 a	29979	4.41a
2	6	33093	4.50 b	12845	4.01b
1	6	13093	4.04 c	7833	3.83bc
0	6	2759	3.26 d	5251	3.66c
lsd (P=0.05)			0.34		0.32

[a] Mean values in a column, for each generation, followed by the same letter do not differ.

resistance to root galling and resistance to nematode reproduction being under the same genetic control. The log-transformed total eggs per root system and eggs per gram of fibrous root in randomly selected F_3 and F_4 families demonstrated a linear trend in regression analysis and significant separations of the egg production levels reflective of F_2 and F_3 parental plant GR scores. These separations provided evidence for three phenotype categories of resistance and susceptibility. Those plants with GR 0 clearly supported very little nematode reproduction and typically produced uniformly resistant families in the next generation through selfing. We found that plants with this level of resistance were often homozygous for the resistance trait. Plants with GR 1 and GR 2 scores demonstrated a somewhat elevated reaction or partial resistance also for egg production. The F_2 and F_3 plants in this category typically produced F_3 and F_4 families that were segregating for resistance, i.e., plants with this level of resistance were heterozygous for the resistance trait. The third category of plants was those with GR 3–GR 8 that had very high levels of egg production on roots, indicating complete susceptibility. They produced uniformly susceptible families upon self-pollination, i.e. these plants were homozygous susceptible for resistance. The egg production data support our GR-based classification of resistance at GR 0–GR 2 and susceptibility for plants with GR 3–GR 8 for use in the genetic model application.

The F_2 and F_3 family distributions observed for the response of carrot to *M. javanica* attack suggested a relatively simply inherited dominant resistance. The simplest model is a single gene but linked duplicate factors in the coupling phase could also explain the observed segregation patterns. General scenarios for a single gene and duplicate factors at 10, 20, 40, and 50 cM (Table 5) indicate that a tighter linkage of 10 or even 20 cM better fits our observations than does a looser linkage. The characteristic distinguishing a single dominant locus from linked duplicate loci is the occurrence of individuals heterozygous for both loci (genotypes C and D, Table 5). These two genotypes yield segregation ratios which are intermediate between the 3:1 and 1:1 ratios expected upon self-pollination and testcrossing, respectively, of heterozygotes; and the 1:0 ratio expected from both selfing and testcrossing homozygous-dominant individuals. For close linkages, the progeny ratios of doubly heterozygous individuals in coupling (genotype C) are similar to those for singly heterozygous individuals (genotype B), while the progeny ratios of doubly heterozygous individuals in repulsion (genotype D) are more similar to those for homozygous dominant individuals (genotype E). A large number of progeny derived from self-pollination or testcrossing are

Table 5 Segregation ratios for linked duplicate factors, coupling phase

Linkage distance (cM)	Population		Genotype[a]					B vs. C[b]	D vs. E[c]
			$\frac{r_1r_2}{r_1r_2} = A$	$\frac{R_1r_2}{r_1r_2} = B$	$\frac{R_1R_2}{r_1r_2} = C$	$\frac{R_1r_2}{r_1R_2} = D$	$\frac{R_{1-}}{R_{1-}} = E$		
		Progeny ratio[f]	(0:1/0:1)	(3:1/1:1)	–	–	(1:0/1:0)	–	–
0	Total[d]		25%	50%	0	0	25%		
	No rec.[e]		–	66.7%	0	0	33.3%		
		Progeny ratio	(0:1/0:1)	(3:1/1:1)	(79.75:20.25/55:45)	(99.75:0.25/95:5)	(1:0/1:0)	(840/1080)	(1197/59)
10	Total		20.25%	9.0%	40.5%	0.5%	29.75%		
	No rec.		–	11.3%	50.8%	0.6%	37.3%		
		Progeny ratio	(0:1/0:1)	(3:1/1:1)	(84:16/60:40)	(99:1/90:10)	(1:0/1:0)	(215/267)	(299/29)
20	Total		16.0%	16.0%	32.0%	2.0%	34.0%		
	No rec.		–	19.0%	38.1%	2.4%	40.5%		
		Progeny ratio	(0:1/0:1)	(3:1/1:1)	(91:9/70:30)	(96:4/80:20)	(1:0/1:0)	(55/63)	(74/14)
40	Total		9.0%	24.0%	18.0%	8.0%	41.0%		
	No rec.		–	26.4%	19.8%	8.8%	45.0%		
		Progeny ratio	(0:1/0:1)	(3:1/1:1)	(93.75:6.25/75:25)		(1:0/1:0)	(36/38)	(47/11)
50	Total		6.25%	25.0%	25.0%	43.75%			
	No rec.		–	26.7%	26.7%	46.7%			

[a] B=R_1r_2/r_1r_2 or r_1R_2/r_1r_2; E=$R_1–/R_1–$ or $–R_2/–R_2$

[b] Minimum number of self / testcross progeny necessary to differentiate between genotypes B and C, *P*=0.05 (Hanson 1959, example C)

[c] Minimum number of self / testcross progeny necessary to identify a recessive (susceptible) segregant, *P*=0.05 (Hanson 1959, example A)

[d] Segregation ratio of the total population derived from a heterozygous individual, coupling phase

[e] Segregation ratio of the population derived from a heterozygous individual, coupling phase, but excluding homozygous recessive progeny (Genotype A)

[f] Segregation ratio of progeny from indicated genotypes as (all dominant genotypes: homozygous recessive for both genes) after self-pollination/test-crossing

needed to differentiate genotypes B and C (215 or 267 if R_1 and R_2 are 20 cM apart, respectively; 840 or 1080 if they are 10 cM apart). Genotype D, resulting from the combination of two single crossover gametes, is especially rare. Even so, the identification of genotype D, which yields a low incidence of homozygous-recessive gametes (susceptibles) is the clearest evidence distinguishing a single locus from linked duplicate loci since recessive progeny cannot be recovered from an individual that is homozygous dominant at a single locus (genotype E); they can, however, be recovered from genotype D. When test-crossed, 29 BC_1 plants need to be scored to discriminate between genotypes D and E if R_1 and R_2 are 20 cM apart, 59 plants if they are 10 cM apart.

Since F_2 infection ratings were known and we sampled F_3 families in this study, the segregation ratios in Table 5 are presented for both the total population and the resistant portion of the population (No rec., Table 5). If no distinction can be made among resistant segregants B, C, D, or E the incidence of genotype D is 0.6% (10 cM) or 2.4% (20 cM). With this, 498 (10 cM) or 124 (20 cM) F_3 testcross families would need to be evaluated to be confident (*P*=0.05) that genotype D does not occur. These values were calculated from example A of Hanson (1959), *n*=(log P) / (log q), where *n*=number of progenies, *P*=level of probability (0.05), and q=probability of failure (0.994 at 10 cM, 0.976 at 20 cM). Therefore, in summary, 124 testcross families of at least 29 plants each would need to be tested to identify genotype D and discrimate it from genotype E if duplicate loci are 20 cM apart. At least 59 plants in 498 families would need to be tested if they are 10 cM apart.

Assuming that a single dominant allele confers complete resistance and applying the model segregation ratios for linkage distances of 0, 10, and 20 cM to the observed ratios, we find that for the F_2 family, duplicate loci 10 cM apart provides the best fit with the observed data (Table 6). The segregation we observed also fits a model of duplicate loci 20 cM apart while a single locus model fails. Presuming a 3.4% error in scoring susceptible F_2 plants as resistant, an acceptable χ^2 is noted with the single-locus (0 cM) and 10-cM model, while the 20-cM model falls below the 1% probability level. Larger linkage distances gave an even poorer fit with our observed results (data not presented).

Since our F_3 families were much smaller than those required to differentiate B versus C and D versus E for loci up to 20 cM apart, it was not possible to classify F_3 families accurately. In fact all segregating F_3 families fit a 3:1 ratio except for those 12 with less than 9% susceptible progeny and those 3 with over 33% susceptible.

F_4 and BC_1 progeny testing was performed for selected resistant and susceptible F_3s in 2 of the 3 families with over 33% susceptible progeny. For both of these families (nos. 422 and 489, Table 2) susceptible (GR 3–8) F_3s yielded uniformly susceptible F_4 families and a small BC_1 family was also susceptible in Family no. 422. Furthermore, some of the resistant F_3s yielded uniformly resistant F_4 families whereas others yielded segregating F_4 families, as would be predicted for a

Table 6 Fit of observed segregation for resistance to *Meloidogyne javanica* in carrot F_2 and F_3 populations to expected ratios (*S* susceptible·*R* resistant·*ns* not significant)

Genotype	Linkage distance (cM):	Expected % in F_2 family[a]			Observed[b]		Expected % in in F_3 families from R–F_ss[a]			Observed % in F_3 families from resistant F_2s					
		0	10	20	*n*	%				Scenario 1[c]			Scenario 2[d]		
							0	10	20	Percentage S grouping	F_3 families *n*	%	Percentage S grouping	F_3 families *n*	%
$\frac{r_1r_2}{r_1r_2} = A$		25	20.25	16.0	90	19.1	–	–	–						
$\frac{R_1r_2}{r_1r_2}$ or $\frac{r_1R_2}{r_1r_2} = B$							66.7	11.3	19.0	3–51	55	64.7			
$\frac{R_1R_2}{r_1r_2} = C$							–	50.8	38.1	–			4-51	53	62.4
		75	79.75	84.0	380	80.9									
$\frac{R_1r_2}{r_1R_2} = D$							–	0.6	2.4	–					
$\frac{R_{1-}}{R_{1-}}$ or $\frac{R_{2-}}{R_{2-}} = E$							33.3	37.3	40.5	0	30	35.4	0–3	32	37.6
χ^2		*n*=470, *df*=1									*n*=85, *df*=1			*n*=85, *df*=1	
0 cM		8.59** (2.41 ns)[e]	–	–							0.15			–	
10 cM		–	0.35 ns (0.79)	–							–			<0.1 ns	
20 cM		–	–	3.46 ns (12.17**)							–			0.97 ns	

*P=0.05–0.10, ** P<0.05

[a] Presuming homozygous recessive genotype A is S (susceptible) and other genotypes (B-E) are R (resistant)

[b] R=gall ratings 0,1,and 2; S=gall ratings 3–8

[c] Scenario l, Single locus, two F_3 progeny classes – uniformly resistant (0% S) and segregating (3–51% S)

[d] Scenario 2, Duplicate loci, four F_3 progeny classes lumped into two categories – Genotype B+genotype C 4–51% S, and genotype D+genotype E, 0–3% S

[e] χ^2 values corrected for 3.4% error in scoring susceptible F_2 plants as resistant

linked-duplicate or single-factor dominant model. While both segregating F_4 families fit 3:1 and their respective backcrosses fit 1:1 in family no. 422, only 4 of 5 F_4 families fit 3:1 in family no. 489. The outlying F_4 family had an excess of susceptible plants, not unlike its F_3 family. Therefore in these 2 families tested with a higher incidence of susceptible F_3 plants, F_4 progeny testing generally upheld a single-factor model for *M. javanica* resistance although a higher incidence of susceptibility was also observed in some F_4 families.

Family no. 192 A, with 18% susceptible progeny, fit 3:1, 79.75:20.25, and 84:16 progeny ratios. An excess of susceptible segregants was also observed in 1 of 9 F_4 families from resistant F_3 plants Three of these families also had a very low incidence (<1–2%) of susceptible progeny, whereas the other F_4 progeny of resistant F_3s fit either 1:0 or 3:1. F_4 progeny from 2 susceptible F_3 segregants fit the expected 0:1, as did a BC_1 for 1 of these F_3s. Thus, this small sampling of family 192 A had many of the properties of the original F_2: segregants fitting a single-factor model along with a few high % S and several very low % segregants.

Twelve F_3 families with less than 9% susceptible progeny failed to fit a 3:1 progeny ratio. In all, 10 of these families did fit the 84:16 and 79.75: 20.25 ratios expected for progenies of genotype C with duplicate resistance loci 20 cM and 10 cM apart, respectively. It was only the 2 families with 3% susceptible which failed to fit the segregation ratios for genotypes B and C (10 cM or 20 cM), but they did fit the ratios for genotype D. F_4 progeny testing was performed for 1 of these family (no. 59), but unfortunately the rare susceptible progenies failed to produce seed so only 4 F_4 progenies from resistant F_3s were evaluated. They were uniformly resistant, as predicted.

Two F_4 families and their BC_1 testcrosses to B6274, the susceptible parent, were generated and tested from the uniformly resistant family no. 112. As expected, all progenies were uniformly resistant.

The single-locus model acceptably fits the F_3 data from resistant F_2s (Scenario 1, Table 6), presuming that

any F_2 with uniformly resistant progeny was homozygous for the resistant locus. The grouping of families with such a broad range of % S, 3–51% when 25% is expected, plus the fact that 12 of these families do not fit the 3:1 ratio, begs a broader consideration of the data with a linked duplicate factor model. The size of families evaluated does not permit a distinction between genotypes B from C, or D from E, but the collective expected ratios of a B+C grouping (3:1 and 79.75:20.25 or 3:1 and 84:16 for 10 and 20-cM linkage, respectively) and a D+E grouping (99.75:0.25 and 1:0 or 99:1 and 1:0 for 10 and 20-cM linkages, respectively) do fit the observed % S groupings of 4–51% and 0–3% in Scenario 2. With this, either a 10-cM or 20-cM linkage distance model fits.

The egg production data in the F_2, F_3 and F_4 families provided supporting evidence for the resistance being expressed at a slightly lower level in the heterozygous condition, i.e., in plants that produced segregating families through selfing. We can conclude that the resistance, while overall expressed in a dominant fashion, does exhibit some gene dosage response with a reduction of resistance in the heterozygous condition. This result is important to consider in the preferred protocol of breeding hybrid carrot cultivars for commercial production.

This research has provided convincing evidence for relatively simply inherited dominant resistance to *M. javanica* in carrot. Field testing in progress indicates that this resistance is very effective against both *M. javanica* and *M. incognita*. To confirm a single-locus model, we need to evaluate at least 52 testcross families of 29 individuals each (20-cM linkage distance) or 229 testcross families of 59 individuals each (10-cM linkage distance) from genotypes D and E-F_3 families. If no susceptible progenies are observed, then we can be confident (P=0.5) that a single locus conditions resistance to *M. javanica* in carrot. Another approach to help confirm a single locus model for resistance is to evaluate linked loci (Boiteux et al. 1999). We have identified four random amplified polymorphic DNA (RAPD) and 11 amplified fragment length polymorphism (AFLP) bands linked to this trait, and work is in progress to characterize flanking markers.

The single dominant locus model is appealing because *M. javanica* resistance in tomato is inherited in this same fashion (Milligan et al. 1998; Roberts et al. 1998). Furthermore, we have found that carrots resistant to *M. javanica* are also resistant to *M. incognita*, similar to resistance in tomato and in several other crop plants (Roberts et al. 1998). With the Mi gene of tomato recently cloned (Milligan et al. 1998), further comparison of these two systems is warranted.

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