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Varietal Response and Estimates of Heritability of Resistance to *Meloidogyne javanica* in Carrots[1]

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Abstract: With methods developed in this study, varietal responses to *M. javanica* were evaluated and heritability of resistance of two promising carrot cultivars was estimated. More egg masses were found on root systems inoculated with eggs added to the soil in three holes in 250 cm^3 cups than by mixing the inoculum with soil in the cups. A resistant breeding line, CNPH 1437, was discriminated from susceptible cultivar Nova Kuroda with inoculum levels higher than 2,000 eggs per cup. Greenhouse and field results suggested that cultivars Nantes Superior and Shin Kuroda were susceptible, Kuronan was somewhat tolerant, and Brasilia and Tropical were resistant to *M. javanica.* Nantes Superior or Shin Kuroda yielded less in carbofuran-treated soil (3 kg a.i./ha) than Kuronan, Brasilia, and Tropical did in nontreated soil. However, incorporation of the nematicide greatly increased yields of Kuronan (32%), Brasilia (62%), and Tropical (91%). Primary root galling at the seedling stage was an adequate parameter for resistance evaluation. Estimated heritability were 0.48 ± 0.07 for primary root galling and 0.35 ± 0.08 for egg mass production in Brasilia, and 0.16 ± 0.11 for primary root galling and 0.31 ± 0.09 for egg mass production in Kuronan.
Key words: carbofuran, *Daucus carota,* root-knot nematode, host response, tolerance.

In Brazil, one of the most important nematode diseases of carrots (*Daucus carota* L.) is caused by *Meloidogyne javanica* (Treub.) Chitwood. Infected roots show galling, forking, and ramification, resulting in low market value. Controlling root-knot in carrots is necessary for profitable production in infested soils (12). One of the most efficient and inexpensive ways to control root-knot nematodes is with varietal resistance. Some carrot cultivars and breeding lines have been tested for resistance to *M. hapla* Chitwood (1,3,13,14), but few have been tested against other *Meloidogyne* species. In a preliminary study, Charchar et al. (2) selected six breeding lines resistant to *M. incognita* (Kofoid and White) Chitwood and *M. javanica.*

This study was done to establish a reliable method for evaluating carrot germplasm for resistance to root-knot nematodes in the greenhouse and to compare the reaction of five carrot cultivars to *M. javanica* with a resistant carrot line (CNPH 1437) selected by Charchar et al. (2) under greenhouse and field conditions. Estimates of heritability for resistance to *M. javanica* were calculated for two promising cultivars, Kuronan (8) and Brasilia (11).

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MATERIALS AND METHODS

Greenhouse experiments

A *M. javanica* population originally increased from one egg mass was maintained on tomato plants (*Lycopersicon esculentum* Mill., cv. Santa Cruz Sakai). Eggs extracted with NaOCl solution from infected roots were used as inoculum (7). Plants were grown in 250-cm^3 plastic cups in methyl bromide-treated soil (39% sand, 22% silt, 39% clay, and pH 6.3). Greenhouse temperatures ranged from 18 to 32 C.

Test 1: Two inoculation methods were compared. In the first method (Central Inoculation Method—CIM), 2,000 eggs were placed in three holes (0.6 cm d and 4 cm deep) near the center of each plastic cup, whereas in the second method (Mixed Inoculation Method—MIM), the eggs were uniformly mixed with soil in the cups. Carrots were seeded in the cup center within 24 hours. There were five replicates of 12 cups each for the resistant line CNPH 1437 and for the susceptible cultivar Nova Kuroda in a randomized split-plot design. Carrots were thinned to two seedlings per cup after emergence. At 45 days, roots were stained with phloxine B (4) and egg masses counted.

Test 2: To determine an adequate inoculum level for germplasm screening, the CIM was applied. In a randomized split-plot design with seven replicates, inoculum levels (0, 1,000, 2,000, 4,000, and 6,000 eggs per cup) served as main plots and cultivars (Nova Kuroda and CNPH 1437) as subplots. Egg masses per root system and galls per primary root were counted 45 days after emergence. Fresh top and root weights were recorded and expressed as percentage of suppression in relation to the noninoculated control.

Test 3: CNPH 1437 and cultivars Nantes Superior, Shin Kuroda, Kuronan, Brasilia, and Tropical were inoculated with 2,000 *M. javanica* eggs per cup by the CIM. Data collected were as in Test 2; treatments were replicated in seven randomized complete blocks.

Test 4: Brasilia and Kuronan, each with 80 half-sib progenies, were evaluated for heritability for resistance to *M. javanica.* This test was conducted as Test 3 and treatments were replicated in three randomized complete blocks. Each half-sib progeny was evaluated 45 days after seedling emergence; the numbers of egg masses per root system and primary root galls were counted. Plot means were used in all genetic and statistical analyses.

Estimates of the genetic parameters for resistance to *M. javanica* were obtained by using variance components according to Kempthorne (9), Vencovsky (10), and Hallauer and Miranda Fo (5). Narrow sense heritabilities and their standard errors were estimated as follows:

$$h^2 = \frac{\text{var HS}}{\frac{\text{var E}}{r} + \text{var HS}},$$

$$SE(h^2) = \left(\frac{2}{n_1 + 2} + \frac{2}{n_2 + 2}\right)^{1/2} \cdot (1 - h^2)$$

Where var HS = half-sib progeny variance, var E = error variance, r = replicate, n_1 and n_2 = degrees of freedom in progenies and errors, respectively.

Field experiments

Test 1: The first test was conducted in loam soil (12% sand, 27% silt, 61% clay, pH 5.0) with an undetectable population level of root-knot nematodes. Egg inoculum was obtained (7) from infected tomato roots in a field. A multiple dibble made from a piece of wood (2 × 6 × 100 cm) and 40 nails was used to make 80-cm rows of holes (each 4 mm d and 6 cm deep, 2 cm apart) in the growing beds. Forty thousand eggs per row were placed in the holes and covered with soil. Carrots were seeded immediately. This test was arranged in a randomized split-plot design with five main plots (the five cultivars) and two subplots (with and without inoculation) and replicated five times. Plants were evaluated for primary root galling 35 days after emergence, and the weight of marketable roots (> 2 cm d and > 9 cm long) was recorded at 106 days.

Test 2: In the second test, experimental plots (1 × 5.0 m each) were established in loam soil (15% sand, 29% silt, 56% clay, pH 4.9) naturally infested with *M. javanica* (206 ± 9 second-stage juveniles/250 cm^3 soil). This test was arranged in a Latin square design with six carrot treatments (CNPH 1437 and the five cultivars) and six replicates. Each plot was split into two subplots (1 × 2.3 m each) with or without carbofuran (Furadan 5 G) treatment at a rate of 3 kg a.i./ha. Carrots were seeded in 11 rows (each 90 cm long, 20 cm apart) per subplot. Data were collected as previously described for the greenhouse tests.

Results

Greenhouse experiments

Test 1: More egg masses were found on root systems inoculated by the CIM than by the MIM (9.7 and 4.3, respectively, $P = 0.05$). More egg masses were found on Nova Kuroda than in CNPH 1437 (12.4 and 1.6, respectively, $P = 0.05$). No interactions between methods and cultivars were observed.

Test 2: Top and root weights of Nova Kuroda and CNPH 1437 were not different ($P = 0.05$) at an inoculum level of 1,000 eggs per cup (Fig. 1). At inoculum levels higher than 2,000 eggs per cup, CNPH 1437 can be differentiated from Nova Kuroda ($P = 0.05$) on the basis of all four parameters.

Test 3: Plant growth suppression was higher in Nantes Superior and Shin Kuroda than in the other cultivars (Table 1). The greatest numbers of egg masses and galls in roots were produced on Nantes Superior, intermediate numbers on Shin Kuroda and Kuronan, and the fewest on Brasilia, Tropical, and CNPH 1437.

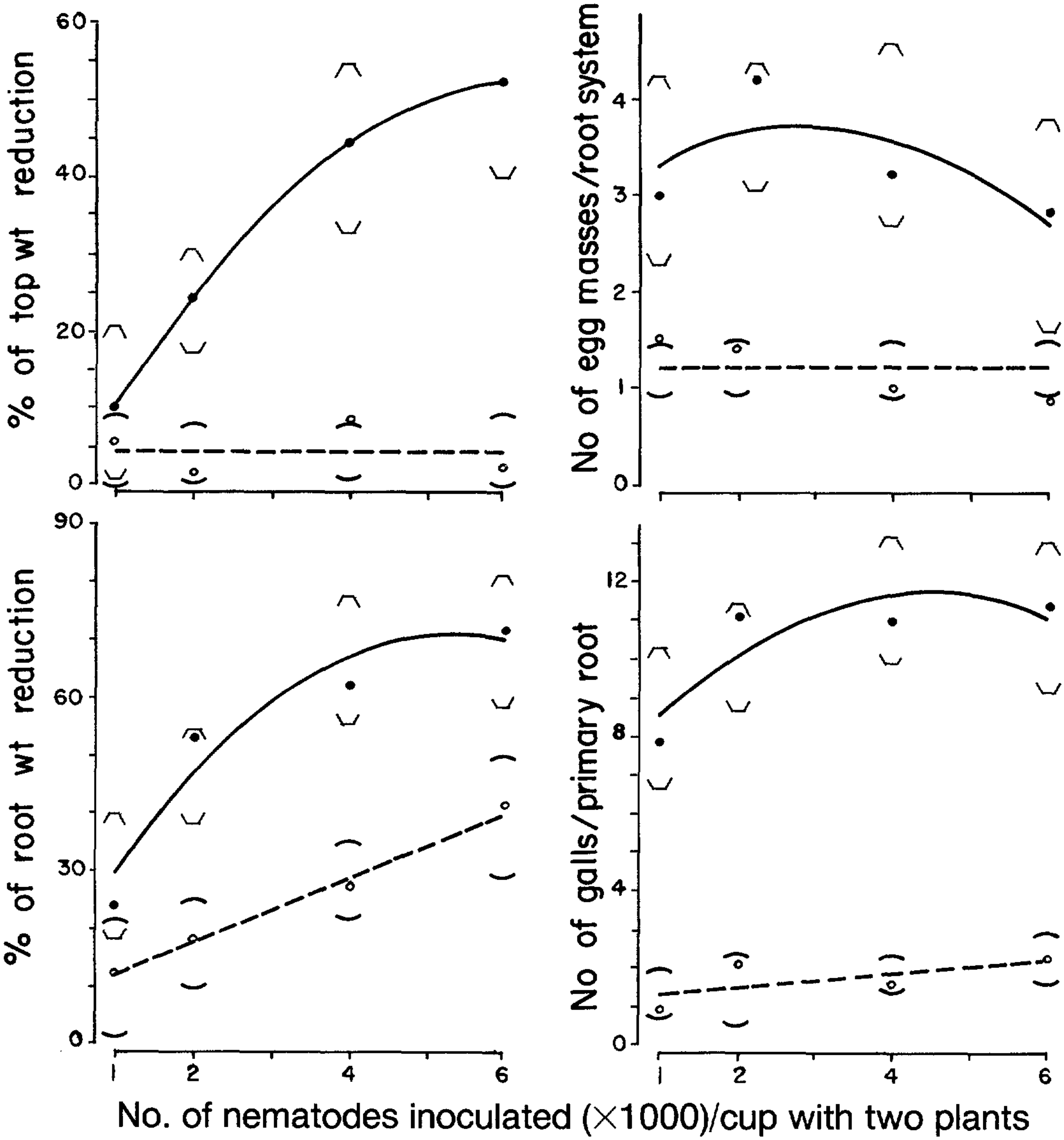

FIG. 1. Reaction of the susceptible cultivar Kova Kuroda (——) and the resistant breeding line CNPH 1437 (– – –) to *M. javanica* at four inoculum levels and four evaluation parameters.

The estimates of the narrow sense heritability for host plant reaction to *M. javanica* are shown in Table 2. The resistance level with the two traits in Brasilia was higher than that in Kuronan. Estimated h^2 for primary root gall and egg mass in Brasilia and for egg mass in Kuronan were within median ranges, but in the low range for primary root galls in Kuronan.

Field experiments

Test 1: Primary root galls were most numerous in Nantes Superior, intermediate on Shin Kuroda and Kuronan, and fewest on Brasilia and Tropical (Table 3). Yield suppression was about 20% in Nantes Superior and Shin Kuroda and 5% in Kuronan and Tropical, but there were no statistical differences among them. Yield was not suppressed in Brasilia.

Test 2: Root-gall number in Kuronan was lower than in Nantes Superior or Shin Kuroda and higher than in Brasilia, Tropical, or CNPH 1437 (Table 4). Numbers of marketable roots were high in Kuronan, Brasilia, and Tropical; moderate in CNPH

TABLE 1. Carrot response to *Meloidogyne javanica* in the greenhouse 45 days after emergence.

Cultivar and line	% of top wt. suppression*	% of root wt. suppression	Egg masses/ root system†	Galls/ primary root
Nantes Superior	77.8 a	82.3 a	6.4 a	5.8 a
Shin Kuroda	76.6 a	75.7 a	4.5 b	3.6 b
Kuronan	40.9 b	43.4 b	4.7 b	3.7 b
Brasilia	26.6 b	34.6 b	3.8 bc	1.8 c
Tropical	34.2 b	28.5 b	2.7 c	2.0 c
CNPH 1437	28.2 b	38.2 b	2.7 c	1.9 c

Means within columns followed by different letters indicate significant difference according to Duncan's multiple-range test ($P = 0.05$).

* Top and root wt. suppression (%) = (1 − weight of nematode-inoculated plant/weight of noninoculated plant) × 100.

† Mean number of egg masses/root system, or galls/primary root, determined by examination of 20 plants/replicate.

TABLE 3. Evaluation of five carrot cultivars artificially inoculated with *Meloidogyne javanica* in field.

Cultivar	Galls/primary root*	% of yield suppression†
Nantes Superior	2.54 a	18.7 a
Shin Kuroda	1.61 b	19.1 a
Kuronan	1.35 bc	5.8 ab
Brasilia	0.83 cd	0.0 b
Tropical	0.62 d	6.4 ab

Means within columns followed by different letters indicate significant difference according to Duncan's multiple-range test ($P = 0.05$).

* Means of five replicates of 100 plants each; evaluated 35 days after emergence.

† Calculated by (1 − marketable root weight from inoculated plot/marketable root weight from control plot) × 100; evaluated 106 days after emergence.

1437; and very low in Nantes Superior and Shin Kuroda. Root weights showed in a decreasing order: Brasilia, Tropical, CNPH 1437, Kuronan, Shin Kuroda, and Nantes Superior. Carbofuran decreased primary root galls in Nantes Superior, Shin Kuroda, and Kuronan, but not in Brasilia, Tropical, and CNPH 1437. Soil treatment with carbofuran increased marketable yields in all cultivars tested and in CNPH 1437. Nematicide-treated Nantes Superior or Shin Kuroda yielded less than untreated CNPH 1437, Tropical, Kuronan, and Brasilia. With carbofuran treatment, however, yield was increased by 32% in Kuronan, 62% in Brasilia, 91% in Tropical, and 109% in CNPH 1437.

DISCUSSION

Galling, forking, and ramification of mature carrot roots have been used to evaluate damage from *M. hapla* (12,13). In our Field Test 1, pronounced galling symptoms on roots were evident 35 days after emergence. Galling remained distinguishable at 70 days but became vague for evaluation after 106 days, indicating that the gall symptom on carrot apparently diminished with age. Also, low incidences of forking and ramification (less than 1%) in mature roots were observed in both field tests. However, primary root galling at seedling stage did differentiate the cultivar response to *M. javanica* in this study. Correlation coefficients among data between primary root galling and mature root yield, and between greenhouse and field conditions with the primary root galling trait, were high (Table 5). The results suggested that primary root galling in the carrot seedling stage is an adequate parameter for resistance evaluation.

Charchar et al. (2) selected CNPH 1437 from four carrot growth cycles in the field for resistance to *M. incognita*. At the end of the fourth cycle, *M. javanica* populations were also found in the field. They concluded that CNPH 1437 was in addition possibly resistant to *M. javanica*. The present study used a single egg-mass isolate of

TABLE 2. Population means (PM), ranges (R), and narrow sense heritabilities (h^2) for resistance to *Meloidogyne javanica* in carrots.

Trait	PM ± SE	R	h^2 (%) ± SE
Cv. Brasilia			
Egg masses/root system	0.54 ± 0.49	0–2.7	35.2 ± 8.4
Galls/primary root	0.60 ± 0.48	0–2.4	48.4 ± 6.7
Cv. Kuronan			
Egg masses/root system	6.53 ± 5.11	1.0–15.3	30.7 ± 9.0
Galls/primary root	3.37 ± 2.00	1.1–5.9	15.6 ± 10.9

TABLE 4. Seedling root-gall numbers and marketable root yields of carrots in *Meloidogyne javanica* infested plots with and without carbofuran at 3 kg a.i./ha.

	Carrot seedling		Marketable root yield			
	No. of galls/primary root†		Number		Weight (g)	
Cultivar and line	Untreated	Treated	Untreated	Treated	Untreated	Treated
Nantes Superior	4.6 a	1.8 a**	8.5 c	45.5 c**	250 f	1,583 f**
Shin Kuroda	6.6 a	1.4 a**	6.5 c	54.0 c**	379 e	3,050 e**
Kuronan	1.2 b	0.7 b*	73.2 a	91.8 b**	4,208 b	5,558 d**
Brasilia	0.2 c	0.1 c	71.0 a	112.8 a**	4,933 a	8,000 a**
Tropical	0.2 c	0.1 c	63.7 a	104.2 a**	3,908 c	7,467 b**
CNPH 1437	0.3 c	0.1 c	53.2 b	85.5 b**	3,233 d	6,767 c**

Means within columns followed by common letters indicate not different according to Duncan's multiple-range test (P = 0.05). Evaluation was made for seedling 35 days and for root yield 100 days after emergence.

† Six replicates of 50 plants each.

* and ** = significantly different from untreated control: $P = 0.05$ and $P = 0.01$, respectively.

M. javanica for greenhouse tests and two natural populations for field tests. Our results confirm the resistance of CNPH 1437 to *M. javanica*. CNPH 1437 was derived from a Brazilian carrot population from which Brasilia and Tropical were selected. In our observations in some *M. incognita*-infested fields, Brasilia and Tropical also showed less severe symptoms than Nantes and Kuroda groups. Thus, it is likely that both Brasilia and Tropical have the same resistant level as CNPH 1437 to *M. javanica* and *M. incognita*.

In comparison, the gall indices on Kuronan seedlings were higher than on Brasilia or Tropical, but weight suppression by the nematode was moderate in Kuronan. Thus, Kuronan is rated as tolerant. Nantes Superior and Shin Kuroda, showing high weight loss and nematode infection, are classified as susceptible. Because of wide distribution of root-knot nematodes and prevalence of Nantes and Kuroda cultivars in commercial cultivation, large carrot losses would be expected in Brazil.

TABLE 5. Correlation coefficients among data between primary root gall and root yield, and between greenhouse and field tests in five cultivars and CNPH 1437 in this study.

	Primary root gall		
Parameter	Greenhouse test 3	Field test 1	Field test 2
Greenhouse test 3: primary root gall	—	0.868	0.706
Field test 1: yield suppression (%)	0.774	0.801	0.938
Field test 2: root weight yield	−0.747	−0.823	−0.921

Carbofuran did not offer effective protection to the two susceptible cultivars. Yields of Brasilia and Tropical, however, were greatly increased with the nematicide application. Therefore, a combination of a nematicide and a resistant cultivar may be required in commercial situations to effectively manage root-knot nematodes.

It is not clear how Kuronan (Nantes × Kuroda Gossum) (8) inherited its moderate reaction to *M. javanica*. Nantes was developed in France and has been traditionally cultivated in Brazil with genetically uniform traits among its commercial cultivars. On the other hand, Kuroda Gossum has Japanese parentage and exhibits resistance to high temperature and to *Alternaria dauci*. In this study, Nantes was susceptible to *M. javanica*. Hence, the tolerance of Kuronan may come from Kuroda Gossum which possibly varies in reaction to root-knot nematodes from other commercial Kuroda cultivars. Further study will be required to determine origin of the Kuronan reaction to *M. javanica*.

Estimated h^2 for two traits indicates that the resistance level of Brasilia to *M. javanica* can be elevated in the processes of recurrent mass selection. It may be possible to increase the resistance level of Kuronan through a series of recurrent selection cycles for the egg mass trait, since it showed a large variation and more genetic variability for the trait. Primary root galling will not be a good trait for resistance selection in Kuronan, because of its low h^2. The nature of resistance has been studied in Brasilia and Kuronan (6).

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