

Inheritance of resistance to the root-knot nematode *Meloidogyne javanica* in lettuce

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ABSTRACT. Resistance to the root-knot nematodes *Meloidogyne* spp. would be a valuable attribute of lettuce *Lactuca sativa* L. cultivars grown in tropical regions. The looseleaf lettuce 'Grand Rapids' is resistant to both *M. incognita* and *M. javanica*. Resistance to *M. incognita* has a high heritability, under the control of a single gene locus, in which the 'Grand Rapids' allele, responsible for resistance (*Me*), has predominantly additive gene action, and has incomplete penetrance and variable expressivity. We studied the inheritance of the resistance of 'Grand Rapids' (P_2) to *M. javanica* in a cross with a standard nematode-susceptible cultivar Regina-71 (P_1). F_1 (Regina-71 x Grand Rapids) and F_2 seed were obtained, and the F_2 inoculated, along with the parental cultivars, with a known isolate of *M. javanica* to evaluate nematode resistance. A high broad sense heritability estimate (0.798) was obtained for gall indices. Class distributions of gall indices for generations P_1 , P_2 , and F_2 were in agreement with theoretical distributions based on a monogenic inheritance model for the range of assumed degrees of dominance between approximately -0.20 and 0.20. *M. javanica* resistance appears to be under control of a single gene locus, with predominantly additive gene action. Whether or not the Grand Rapids allele imparting resistance to *M. javanica* is the same *Me* allele imparting resistance to *M. incognita* remains to be determined.

Key words: *Lactuca sativa* L., Lettuce, Resistance, *Meloidogyne javanica*, Nematode

INTRODUCTION

Varietal resistance is considered one of the most efficient methods for root-knot nematode (*Meloidogyne* spp.) control (Ferraz and Mendes, 1992; Maluf, 1997); however, little emphasis has been placed on breeding lettuce for nematode resistance. In Brazil, both *M. javanica* and *M. incognita* are widespread (Lordello, 1984) and are known to occur sympatrically. Multiple resistance to both species and their races (in the case of *M. incognita*) is an important breeding goal. In lettuce, root-knot nematodes were not recognized as a serious problem until recently (Campos, 1995). Reports of nematode attacks have become more common with increasing lettuce acreages grown under plastic tunnels, leading to increases in nematode populations. The use of susceptible crisphead cultivars, whose cycle is usually much longer than that of traditionally grown butterhead or looseleaf cultivars, has also impacted on nematode populations.

Lettuce breeding practices in Brazil have emphasized selection of genotypes with good vegetative growth under high temperatures, and resistance to the lettuce mosaic virus (LMV) (Nagai and Costa, 1972, 1973; Costa and Silva, 1976; Nagai, 1979, 1980, 1983; Nagai and Lisbão, 1980; Silva et al., 1999). Resistance to *Meloidogyne* spp. in lettuce received little or no attention in the past, but is now considered an important goal in breeding lettuce for both field and greenhouse production in Brazil (Mendes, 1998; Gomes, 1999).

There is little published on varietal resistance to *Meloidogyne* spp. in lettuce. Ryder's comprehensive review of lettuce genetics and breeding (Ryder, 1986) does not report the existence of root-knot nematode resistant lettuce germplasm. Kaloo (1988) reviewed sources of *Meloidogyne* resistance in vegetable crop species, but made no reference to lettuce. An evaluation of the USDA and the University of California *Lactuca* spp. germplasm collections (McGuire et al., 1993) similarly made no reference to sources of resistance to root-knot nematodes. Charchar (1991) was apparently the first to report *Meloidogyne* resistance in lettuce. This report was later confirmed by Gomes et al. (1996), Charchar and Moita (1996), and Mendes (1998). Resistance to both *M. incognita* (races 1, 2, 3 and 4) and *M. javanica* was found in the Grand Rapids cultivar (Mendes, 1998; Gomes, 1999).

Gomes (1999) found that the resistance of the lettuce cultivar Grand Rapids to *M. incognita* has high heritability, and is under control of a single gene locus in which the resistance allele shows incomplete penetrance, variable expression and predominantly additive action. A symbol (*Me*) was proposed for the cultivar Grand Rapids allele that imparts resistance to *M. incognita*, but inheritance of this resistance to *M. javanica* has not been studied. We studied the genetic control of resistance to the root-knot nematode species *M. javanica* in the lettuce cultivar Grand Rapids.

MATERIAL AND METHODS

Plant material

Two lettuce cultivars - Grand Rapids and Regina-71, with contrasting reactions to root-knot nematodes, were used in this study. Regina-71 is a Brazilian white-seeded butterhead cultivar previously characterized as susceptible to both *M. incognita* and *M. javanica* (Gomes et al., 1996; Mendes, 1998; Gomes, 1999). Grand Rapids is a black-seeded looseleaf American cultivar, previously found to be resistant to both root-knot nematode species (Gomes et al., 1996; Mendes, 1998; Gomes, 1999).

Crosses between Regina-71 (seed parent) and Grand Rapids (pollen parent) were made

in accordance with standard emasculation and pollination techniques (Nagai, 1980; Ryder, 1986). Seed color was used as a marker to unequivocally identify true F_1 plants (Ryder, 1986): putative F_1 plants were grown to seed production, and any white-seeded seed parent contaminant plants were eliminated, leaving only true F_1 plants from which F_2 seed were secured. Plants of parental generations P_1 (= Regina-71), P_2 (= Grand Rapids), F_2 (Regina-71 x Grand Rapids) were used to study the inheritance of nematode resistance. The F_1 (Regina-71 x Grand Rapids) generation was not included in the trial, due to an insufficient supply of F_1 seed.

Inoculum preparation and inoculation methods

A known isolate of *M. javanica* (characterized at the Department of Plant Pathology, Universidade Federal de Lavras, Lavras, MG, Brazil) was routinely maintained on isolated benches within plastic houses at the Vegetable Research Station of HortiAgro Sementes Ltda., Ijaci, MG, Brazil. Tomato plants of the susceptible cultivar Santa Clara were grown in 8-liter pots and inoculated with 10,000 nematode eggs/pot. Nematode inocula were prepared in accordance with the technique described by Hussey and Barker (1973), modified by Boneti (1981). Approximately 70 days after inoculation in tomato plants, nematode eggs were extracted by the same technique, and used as inocula for the lettuce plants. A 50% v/v mixture of commercial substrate (Plantimax[®]) + carbonized rice husks was inoculated with the nematode egg suspension, homogenized to a final concentration of 30 eggs per ml mixture, and used to fill styrofoam speedling trays with trapezoidal cells (35 ml substrate per cell). Lettuce seedlings at the one-true-leaf stage were transplanted into the trays (one seedling/cell), where they were kept until the date of resistance evaluation.

Evaluation of plant reactions to *M. javanica*

The evaluation date was chosen based on the presence of abundant numbers of galls in the root system of plants of the susceptible cultivar Regina-71. Inoculated lettuce plants grown in speedling cells had their root systems washed in tap water before scoring for gall indices (GI). GI were assessed on a scale of 1 to 5, based on the number of galls per root system and average gall diameter, as follows: 1 = no galls, or sparse galls with average gall diameter ≤ 1 mm; 2 = sparse galls, with average gall diameter between 1 and 2 mm; 3 = galls mostly non-coalescent, average gall diameter between 2 and 3 mm; 4 = galls numerous and coalescent, average gall diameter between 3 and 4 mm, and 5 = galls numerous and coalescent, average gall diameter >4 mm.

Resistance trials with parental and F_2 generations

A resistance trial was carried out with the parental cultivars Regina-71 (= P_1) and Grand Rapids (= P_2), and the segregating F_2 (Regina-71 x Grand Rapids) generation from November 1998 through February 1999. Plants were distributed in 8-plant plots, whose positions were assigned to the trays in accordance with a completely randomized design. The total number of plants evaluated (final stand) was 99, 118 and 484, for P_1 , P_2 and F_2 , respectively. Inoculation procedures were as previously described. Plant reactions to the nematode were evaluated 70 days after transplanting/inoculation. During the period prior to evaluation of nematode resistance,

plant trays were kept in a plastic house suspended by their borders on metal rails 45 cm above the floor, with cell drainage holes unimpeded.

Estimation of genetic parameters

GI means and variances were calculated for each of the populations in each trial. Genetic (σ_G^2), environmental (σ_E^2) and phenotypic (σ_P^2) variances, as well as broad sense heritability were calculated as indicated by Mather and Jinks (1977). Environmental variance (σ_E^2) was estimated as the geometric mean of total variances in generations P_1 and P_2 . Generation mean analyses were performed on P_1 , P_2 and F_2 (Mather and Jinks, 1977), to estimate the additive [**a**] and non-additive [**d**] mean components and the mean degree of dominance ($MDD = [d] / [a]$).

Test of the hypothesis of monogenic inheritance

Plant frequency distributions of GI were obtained for each generation studied in the trials. An arbitrary truncation point (TP) was chosen to permit a clear discrimination between the parental phenotypes: the TP value corresponded to a GI value below which fell most of the plants of the resistant parent, and above which fell most of the plants of the susceptible parent.

Individual plant phenotypes were not classified into discrete classes (resistant/susceptible) because of the continuous nature of the variables under study. Data on the means and variances of P_1 and P_2 were used to calculate expected frequencies of plants below the TP in these populations, as well as in the F_2 , assuming monogenic inheritance and different presumed degrees of dominance.

For each of the different presumed degrees of dominance, the monogenic hypothesis tested was based on the following suppositions and procedures:

- a) The GI follows a Poisson distribution.
- b) Parental generations P_1 and P_2 are assumed to have expected means (and variances) equal to their respective observed means. Frequencies of plants for which the GI value was below or equal to the truncation point ($\leq TP$) were obtained from the theoretical Poisson distribution assumed for P_1 and P_2 , and were taken to represent the respective expected frequencies under the proposed model.
- c) The mean of the F_1 population was taken as:

$$\bar{F}_1 = (\bar{P}_1 + \bar{P}_2)/2 + MDD \cdot (\bar{P}_1 - \bar{P}_2)/2$$

where: \bar{P}_1 and \bar{P}_2 are the respective parental means, and MDD is the mean degree of dominance under consideration. The variance of the F_1 population was taken (under the Poisson distribution) as:

$$V(F_1) = \bar{F}_1$$

d) The frequency of F_1 individuals with GI values $\leq TP$ was estimated from the Poisson distribution assumed for that population, and was assumed to be the true (expected) frequency under the model.

e) Under the hypothesis of monogenic inheritance, the expected frequency of plants in F_2 with GI values $\leq TP$ was calculated as the weighted average of the expected frequencies in

P_1 , P_2 and F_1 (weights 1:2:1, respectively), as estimated in b and d.

f) Expected numbers of plants with GI values \leq TP in P_1 , P_2 and F_2 were calculated by multiplying the expected frequencies (obtained in b, b and respectively) by the total number of plants tested per generation.

g) Expected numbers of plants with values \leq TP in P_1 , P_2 and F_2 were compared with their respective observed values in each generation, and the significance of the deviations was estimated with a χ^2 test. The frequency of expected plants in P_1 was added to that of P_2 , in order to avoid expected cell frequencies equal to zero. The number degrees of freedom in the χ^2 test was therefore 1.

h) χ^2 Values significant at $\alpha = 0.05$ would lead to the rejection of the hypothesis of monogenic inheritance under the degree of dominance considered. On the other hand, a nonsignificant value of χ^2 would not allow rejection of that hypothesis. Values of the χ^2 for each monogenic hypothesis/presumed MDD tested were plotted against their respective hypothetical MDD's. The range of MDD values for which χ^2 values fell below the critical $\alpha = 0.05$ value represented the MDD range for which the monogenic hypothesis was not rejected.

RESULTS AND DISCUSSION

The genetic variance estimate of nematode resistance measured by GI was much higher than the environmental variance, and broad-sense heritability was high (Table 1), indicating that GI is a trait subjected to a low degree of environmental influence. The MDD estimate was -0.3333, indicating a low degree of incomplete dominance in the direction of lower GI. This MDD point estimate, close to zero, indicates a predominantly (but not entirely) additive gene action of the gene(s) controlling nematode resistance - a result similar to that found by Gomes (1999) for the gene that controls resistance to *M. incognita*.

Table 1. Reaction of lettuce cultivars Regina-71 (= P_1), Grand Rapids (= P_2), and F_2 ($P_1 \times P_2$) to inoculations with an isolate of *Meloidogyne javanica*: estimates of phenotypic (σ^2_p), genetic (σ^2_G) and environmental (σ^2_E) variances, broad-sense heritabilities (H^2) and mean degree of dominance for gall index.

Parameter	Estimate
Phenotypic variance (σ^2_p)	1.0033
Genetic variance (σ^2_G)	0.8006
Environmental variance (σ^2_E)	0.2027
Broad-sense heritability (H^2)	0.7980
Mean degree of dominance (MDD)	-0.3333

The high value obtained for the broad-sense heritabilities indicated that response to *M. javanica* in cultivar Grand Rapids is under the control of a single gene locus. However, the frequency distribution of GI in the F_2 generation does not provide clear evidence for monogenic control (Table 2). Phenotypes could not be classified into the discrete classes of resistant or susceptible, due to the continuous nature of the variable under study. This property was associated with the predominantly additive gene action for the trait, which was responsible for the existence in F_2 of GI's intermediary between those of the parents.

The proposed test of the monogenic hypothesis indicated nonetheless that frequency distributions of GI were consistent with segregation at a single locus. Within the degrees of dominance of -0.2 and -0.2 for segregation of a putative locus, the calculated χ^2 values fell below that of the $\alpha = 0.05$ limit (Figure 1). These results strongly support the hypothesis of

Table 2. Reaction of lettuce cultivars Regina-71 (= P₁), Grand Rapids (= P₂) and F₂ (P₁ × P₂) to inoculations with an isolate of *Meloidogyne javanica*: frequency distributions per gall index (GI) class.

Generation	Observed number of plants with gall index				
	GI = 1	GI = 2	GI = 3	GI = 4	GI = 5
Regina-71 (= P ₁)	0	0	8	32	59
Grand Rapids (= P ₂)	105	12	0	0	0
F ₂ (P ₁ × P ₂)	75	166	174	50	19

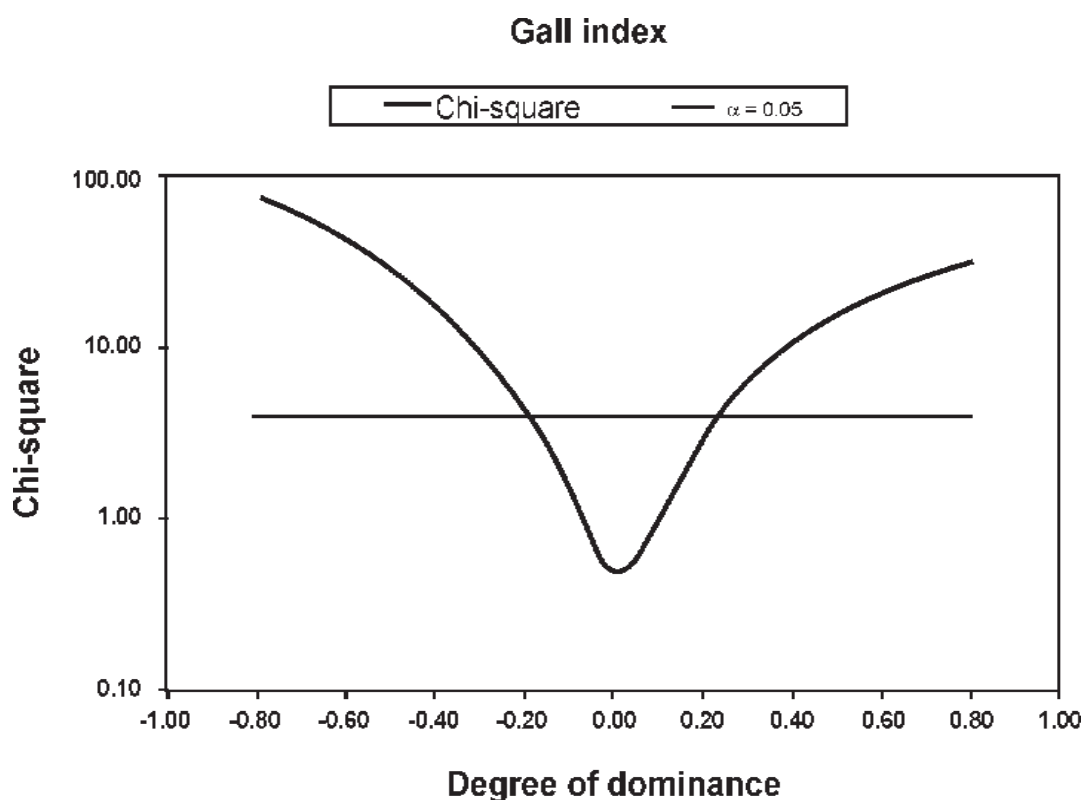


Figure 1. Reaction of lettuce cultivars Regina-71, Grand Rapids and of the population F₂ (Regina-71 × Grand Rapids) to inoculation with an isolate of *Meloidogyne javanica*: test of the monogenic hypothesis under different presumed degrees of dominance.

monogenic inheritance. The phenotypic distributions in the parental and F₂ generations may therefore be explained by the action of a single locus with predominantly additive gene action.

The point estimate for the degree of dominance (Table 1) fell into a range close to the boundaries for degree of dominance within which the null hypothesis of monogenic control of resistance would not be rejected (Figure 1).

These results provide evidence that a single gene locus, or at least a single chromosome segment, may be responsible for resistance to *M. javanica* in lettuce. In spite of the fact that there is clear evidence for one major locus controlling nematode resistance, the data do not

preclude the existence of other genes (modifiers) with minor effects. Our conclusions are similar to those of Gomes (1999), who examined resistance to *M. incognita* in the same lettuce populations. The major allele involved in the *M. javanica* resistance of the lettuce cultivar Grand Rapids bears similarity of gene action to the *Me* allele responsible for resistance to *M. incognita* in the same cultivar. It is therefore possible that the *Me* allele itself could be responsible for resistance to both root nematode species. Our data, however, do not provide evidence to support or invalidate that conclusion. Consequently, the identity between the Grand Rapids alleles for resistance to both nematode species remains to be demonstrated or disproved in further trials.

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REFERENCES

- Boneti, S.I.S. (1981). Inter-relacionamento de micronutrientes como parasitismo de *Meloidogyne exigua* em mudas de cafeeiro (*Coffea arabica* L.). M.S. Dissertation, Universidade Federal de Viçosa, Viçosa, MG, Brazil.
- Campos, V.P. (1995). Doenças causadas por nematóides em alcachofra, alface, chicória, morango e quiabo. *Inf. Agropecu.* 17 (182): 17-22.
- Charchar, J.M. (1991). Comportamento de cultivares de alface à infecção por nematóides de galhas. *Hortic. Bras.* 9: 35 (Abstract).
- Charchar, J.M. and Moita, A.W. (1996). Reação de cultivares de alface à infecção por misturas populacionais de *Meloidogyne incognita* raça 1 e *M. javanica* em condições de campo. *Hortic. Bras.* 14: 185-189.
- Costa, C.P. and Silva, N. (1976). Melhoramento da alface para resistência múltipla ao calor e ao mosaico. *Rev. Oleric.* 15: 26-27.
- Ferraz, S. and Mendes, M.L. (1992). O nematóide das galhas. *Infor. Agropecu.* 16 (172): 43-45.
- Gomes, L.A.A. (1999). Herança da resistência da alface (*Lactuca sativa* L.) cv. Grand Rapids ao nematóide de galhas *Meloidogyne incognita* (Kofoid and White) Chitwood. Doctoral thesis, Universidade Federal de Lavras, Lavras, MG, Brazil.
- Gomes, L.A.A., Mendes, W.P., Maluf, W.R., Azevedo, S.M., Freitas, J.A. and Moretto, P. (1996). Resistência de cultivares de alface à infecção por *Meloidogyne incognita* (raças 1, 2 e 3). *Hortic. Bras.* 14: 87 (Abstract).
- Hussey, R.S. and Barker, K.R. (1973). A comparison of methods collecting inocula of *Meloidogyne* spp. including a new technique. *Plant Dis. Rep.* 57: 1025-1028.
- Kaloo, D. (1988). *Vegetable Breeding* Vol. II. CRC Press, Boca Raton, FL, USA.
- Lordello, L.G.E. (1984). *Nematóides das Plantas Cultivadas*. Nobel, São Paulo, SP, Brazil.
- Maluf, W.R. (1997). Resistência a nematóides de galhas *Meloidogyne* spp. em espécies olerícolas. In: *Resistência de Plantas a Doenças* (Zambolin, L. and Ribeiro-do-Vale, F.X., eds.). Fitopatologia Brasileira/Congresso Brasileiro de Fitopatologia, 30, Palestras, pp. 57-63.
- Mather, K. and Jinks, J.L. (1977). *Introduction to Biometrical Genetics*. Cornell University Press, Ithaca, NY, USA.
- McGuire, P.E., Ryder, E.C., Michelmore, R.W., Clark, R.L., Antle, R., Emery, G., Hannan, R.M., Kesseli, R.V., Kurtz, E.A., Ochoa, O., Rubatzky, V.E. and Waycott, W. (1993). *Genetic resources of lettuce and Lactuca species in California - an assessment of the USDA and UC collections and recommendations for long-term security. Genetic Resources Conservation Program, Division of Agriculture and Natural Resources, University of California, Davis, CA, USA, Report No. 12.*
- Mendes, W.P. (1998). Hospedabilidade e resistência de cultivares de alface (*Lactuca sativa* L.) aos nematóides de galhas *Meloidogyne incognita* (raça 1,3 e 4) e *Meloidogyne javanica*. M.S. Dissertation, Universidade Federal de Lavras, Lavras, MG, Brazil.
- Nagai, H. (1979). Obtenção de novos cultivares de alface (*Lactuca sativa* L.) resistente ao mosaico e ao calor - Brasil

- 48, 202 e 221. *Rev. Oleric. 17*: 129-137.
- Nagai, H.** (1980). Obtenção de novos cultivares de alface (*Lactuca sativa* L.) resistente ao mosaico e ao calor - Brasil-303 e 311. *Rev. Oleric. 18*: 14-21.
- Nagai, H.** (1983). Caracterização de resistência ao calor em alface (*Lactuca sativa* L.). In: *Congresso Brasileiro de Olericultura, 23. Resumos. Sociedade de Olericultura do Brasil, Vitória, ES, Brazil.*
- Nagai, H. and Costa, A.S.** (1972). Resistência ao calor e ao mosaico numa variedade nova de alface tipo manteiga. *Rev. Oleric. 12*: 30 (Abstract).
- Nagai, H. and Costa, A.S.** (1973). Seleção de novas linhagens de alface resistentes ao mosaico e ao calor. *Rev. Oleric. 13*: 27-28.
- Nagai, H. and Lisbão, R.S.** (1980). Observação sobre resistência ao calor em alface (*Lactuca sativa* L.). *Rev. Oleric. 18*: 7-13.
- Ryder, E.J.** (1986). Lettuce breeding. In: *Breeding Vegetable Crops* (Basset, M. ed.). AVI Publishing Co., Westport, CT, USA, pp. 433-474.
- Silva, E.C., Maluf, W.R., Leal, N.R. and Gomes, L.A.A.** (1999). Inheritance of bolting tendency in lettuce *Lactuca sativa* L. *Euphytica 109*: 1-7.