

Inheritance of resistance to *Meloidogyne incognita* race 2 in the hot pepper cultivar Carolina Cayenne (*Capsicum annuum* L.)

Fausto de Souza-Sobrinho¹, Wilson Roberto Maluf², Luiz A.A. Gomes¹
and Vicente Paulo Campos³

Departamentos de ¹Biologia, ²Agricultura, and ³Fitopatologia,
Universidade Federal de Lavras, Caixa Postal 37, 37200-000 Lavras, MG,
Brazil

Corresponding author: W.R. Maluf

E-mail: wrmaluf@ufla.br

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ABSTRACT. Root-knot nematodes of the genus *Meloidogyne* are important pathogens affecting vegetable crop production in Brazil and worldwide. The pepper species *Capsicum annuum* includes both hot and sweet peppers; very little emphasis has been placed on breeding sweet peppers for nematode resistance. We report on the inheritance of resistance to *Meloidogyne incognita* (Kofoid & White) Chitwood race 2 in the hot pepper cultivar Carolina Cayenne. The hot pepper cv. Carolina Cayenne was used as seed parent and the sweet pepper cv. Agronômico-8 was used as pollen parent to obtain the F₁ and F₂ generations and the backcross generations BC₁₁ and BC₁₂. The plants were inoculated with *M. incognita* race 2 at a rate of 60 eggs/ml of substrate and, after a suitable incubation period, the numbers of root galls and egg masses per root system were evaluated on each plant. Broad- (0.77 and 0.72) and narrow-sense (0.77 and 0.63) heritability estimates were high for both root galls and egg masses, respectively. The mean degree of dominance was estimated as 0.29 and 0.25 for numbers of galls and egg masses, respectively; these estimates were not significantly different from 0, indicating a predominantly additive gene action. The results were

consistent with a hypothesis of monogenic resistance in Carolina Cayenne.

Key words: *Capsicum annuum*, *Meloidogyne incognita*, Heritability, Gene action, Monogenic inheritance, Resistance

INTRODUCTION

While most sweet pepper *Capsicum annuum* L. cultivars are resistant to the root-knot nematode species *Meloidogyne javanica*, they are usually susceptible to the Southern root-knot nematode *M. incognita* (Taylor and Sasser, 1978; Peixoto, 1995). Even though varietal resistance is considered one of the most efficient methods for nematode control (Ferraz and Mendes, 1992), very little emphasis has been placed on breeding peppers for nematode resistance. In Brazil both *M. javanica* and *M. incognita* are widespread (Lordello, 1984) and occur sympatrically, so multiple resistance to both species and, in the case of *M. incognita*, to its races, is an important goal for plant breeders.

Several authors have studied the inheritance of the resistance reactions of *C. annuum* lines to root-knot nematodes. Hare (1956, 1957) demonstrated that a dominant gene (*N*) present in cv. Santaka controlled resistance to three different *Meloidogyne* species, but its efficiency depended upon the nematode isolate and the amount of inoculum (Hare, 1957). This gene was reportedly introduced into several commercial cultivars, including Mississippi Nemaheart (Hare, 1957; Fery and Dukes, 1996), but produced little or no resistance against several nematode populations (Di Vito and Saccardo, 1978).

Hendy et al. (1983) identified two pungent *C. annuum* lines, PM-217 (from Central America) and PM-687 (from India) that were resistant to three of the main *Meloidogyne* species, i.e., *M. arenaria* (Neal) Chitwood, *M. incognita* (Kofoid & White) Chitwood and *M. javanica* (Treb) Chitwood. These two *C. annuum* accessions contrast with many cultivars that are susceptible to all three nematode species, and with cv. Yolo Wonder, which is resistant to *M. javanica* and to some populations of *M. arenaria* but susceptible to *M. incognita* (Hendy et al., 1983).

Hendy et al. (1985) studied the inheritance of nematode resistance in PM-217 and PM-687 in crosses with cv. Yolo Wonder, and found two genes (*Me1* & *Me2*) controlling nematode resistance in PM-217, two genes (*Me3* & *Me4*) in PM-687 and one gene (*Me5*) in cv. Yolo Wonder. In PM-217, the *Me1* gene imparts resistance to *M. arenaria*, to *M. incognita* and to *M. javanica* but not the 'Seville' isolate of *Meloidogyne* sp., while the *Me2* gene confers resistance to *M. javanica* and to the 'Seville' isolate. In the PM-687 the *Me3* gene imparts resistance to most *M. arenaria* isolates (except the Ain Toujdate isolate) as well as to *M. incognita* and *M. javanica*, while the *Me4* gene provides resistance to the Ain Toujdate isolate. In the cv. Yolo Wonder the *Me5* gene imparts resistance only to *M. javanica*.

Peixoto (1995) reported that all of the sweet pepper cultivars tested, which included some of the cultivars most widely grown in Brazil, were resistant to *M. javanica*, probably due to the ubiquitous presence of the *Me5* gene, but none of these commercial cultivars were resistant to *M. incognita*. Peixoto (1995) also suggested that either *Me1* or *Me3* might be used for pepper breeding programs in Brazil, where both *M. incognita* and *M. javanica* are recognized as problems, and they demonstrated that advanced breeding lines bearing the *Me1* or *Me3* gene had excellent levels of resistance to races 1, 2, 3 and 4 of *M. incognita*, as well as to *M. javanica*.

Due to the variability that exists within the species *M. incognita*, the search for new *C. annuum* genes conferring resistance to *M. incognita* can still be regarded as priority. Zamora et al. (1994) have described the resistance reaction of cv. Carolina Cayenne to isolates of the four known *M. incognita* races, but no studies of the genetic basis of this resistance were reported in this study. Fery and Dukes (1996) reported that resistance to *M. incognita* race 3 in cv. Carolina Hot (from which cv. Carolina Cayenne was derived) is under the control of two gene loci, consisting of one dominant allele, allelic to the *N* gene present in cv. Mississippi Nemaheart, and another recessive allele. This accounts for the higher resistance found in the homozygous cv. Carolina Hot relative to cv. Mississippi Nemaheart.

A knowledge of the number of genes involved in the control of nematode resistance and the mean degree of dominance of these genes, would contribute to pepper-breeding programs, especially those aimed at developing F_1 hybrids where complete dominance would be preferred. We report the inheritance of resistance to *M. incognita* race 2 in the hot pepper cultivar Carolina Cayenne, and the magnitude of additive and nonadditive genetic effects and the mean degree of dominance of the gene(s) controlling the traits associated with resistance.

MATERIAL AND METHODS

Plant material

Two contrasting *C. annuum* cultivars were used as parents in a cross: cv. Agronômico-8, a Brazilian sweet pepper cultivar reported by Peixoto (1995) as being susceptible to *M. incognita*, and cv. Carolina Cayenne, an American hot pepper cultivar reported by Zamora et al. (1994) as being resistant to races 1, 2, 3 and 4 of *M. incognita*. F_1 hybrid seeds were obtained by crossing Agronômico-8 (P_1) as seed parent to Carolina Cayenne (P_2). F_1 plants were self-pollinated to obtain the F_2 generation and simultaneously backcrossed (BC) as seed parents to both P_1 and P_2 to obtain the BC_{11} and BC_{12} generations, respectively.

Nematode inoculum preparation and inoculation procedures

A known isolate of *Meloidogyne incognita* race 2 (provided by the Instituto Agronômico do Paraná (IAPAR), Londrina, PR, Brazil) was routinely maintained in susceptible tomato (*Lycopersicon esculentum* Mill.) cv. Rey de Los Tempranos plants. When required for inoculation, nematode eggs were extracted from galled tomato roots according to the technique of Hussey and Barker (1973), and an aqueous suspension thoroughly mixed with an artificial substrate (50% commercial substrate Plantimax® + 50% carbonized rice husks) to a final concentration of 60 eggs/ml of substrate.

C. annuum seeds from the P_1 and P_2 parents and the F_1 , F_2 , BC_{11} , and BC_{12} generations were sown in germination trays filled with uninoculated substrate. Sixteen days later (January 22, 1997), seedlings were transplanted into 24-plant plots, using a completely randomized design, and to 128-cell Styrofoam seedling trays filled with the inoculated substrate described above. Since the volume of each pyramidal cell was 35 ml, each plant received about 2100 nematode eggs. Ninety days after inoculation the plants were removed from the trays, their roots washed to remove excess substrate, the nematode egg masses stained with 150 mg/l Phloxine B (Taylor and Sasser, 1978) and the number of galls and egg masses per root recorded.

Statistical design and analyses

Forty-eight plants were assessed for each parent plant (P_1 , P_2) and 72 plants for each of the F_1 , F_2 , BC_{11} and BC_{12} generations, with the means and variances of the number of galls and egg masses per root system being calculated for each group. Additive, nonadditive and environmental variances, broad- and narrow-sense heritabilities and their respective standard errors, as well as the estimated number of loci involved in trait expression were calculated, as indicated by Ramalho et al. (1989) and Vencovsky and Barriga (1992). Generation mean analyses were also performed (Rowe and Alexander, 1980).

Monogenic inheritance testing

Because the numbers of galls and egg masses are continuous variables following Poisson distributions, classification of phenotypes into discrete classes (resistant or susceptible) was not used in this study. Instead, the means and variances of the number of galls and egg masses on P_1 and P_2 plants were used in a model to simulate the expected resistant and susceptible frequencies in both these and the F_1 , F_2 and backcross generations, under the hypothesis of monogenic inheritance and differing degrees of dominance.

For both galls and egg masses, simulations were carried out for different presumed degrees of dominance assuming monogenic inheritance.

The number of galls and egg masses conformed to a Poisson distribution and the estimated \bar{p}_1 and \bar{p}_2 means were assumed to be equal to their respective variances (environmental variances). For each parent plant (P_1 and P_2), 10^4 phenotypes were simulated, assuming a normal distribution for each parent, with the true mean and variance being equal to the observed estimate of their respective means. The frequency of plants with <8 galls (<10 egg masses) in the P_1 and P_2 generations was obtained from the simulated populations and was assumed to be the true (expected) frequencies for the model. The mean and variance of the F_1 generation were considered to be equal to: $(\bar{p}_1 + \bar{p}_2)/2 + MDD(\bar{p}_1 - \bar{p}_2)/2$, where \bar{p}_1 and \bar{p}_2 are the parental means and MDD is the mean degree of dominance under consideration. The same number of simulations (10^4) and the same assumptions regarding normal distribution and the relationship between the mean and variance used for the parental generation was also applied to the F_1 generation. The frequency of F_1 plants <8 galls (<10 egg masses) was recorded and assumed to be the true (expected) frequency for the model. Under the hypothesis of monogenic inheritance the frequency of plants with <8 galls (<10 egg masses) in the F_2 generation was taken as the weighted average of the expected frequencies, $\bar{p}_1 + 2\bar{f}_1 + \bar{p}_2$. In the backcrosses, the frequencies were $\bar{p}_1 + \bar{f}_1$ for BC_{11} and $\bar{p}_2 + \bar{f}_1$ for BC_{12} . The expected frequencies of plants with <8 galls (<10 egg masses) obtained from P_1 , P_2 , F_1 , F_2 , BC_{11} and BC_{12} were multiplied by the total number of plants evaluated per generation to obtain the expected number of plants with <8 galls (<10 egg masses) in each generation. This was compared to the observed number and a χ^2 value calculated with 5 degrees of freedom. For the degree of dominance under consideration a significant χ^2 value signified rejection of the monogenic inheritance hypothesis while a nonsignificant χ^2 value signified non-rejection.

RESULTS AND DISCUSSION

Both the variance components (Table 1) and mean component (Table 2) estimates indi-

cate that the resistance reaction to root-knot nematodes is controlled by gene(s) with predominantly additive gene action. For both galls and egg masses the estimates of nonadditive variances ($\hat{\sigma}_D^2$) were lower than those of additive variances ($\hat{\sigma}_A^2$), and the magnitude of the standard errors of $\hat{\sigma}_D^2$ was zero (Table 1). The estimates of the additive mean component (a) were higher than those of the nonadditive (d) component (Table 2), resulting in MDD estimates of 0.29 for the number of galls and 0.25 for the number of egg masses, indicating very weak or no dominance, favoring larger numbers of galls and egg masses.

Broad-sense heritability for both galls and egg masses was high (>0.70), as was narrow-sense heritability (>0.60) (Table 1), indicating that selection for nematode resistance in the peppers studied would be efficient. High heritability values can indicate that the trait under consideration is under the control of one or a few gene loci. In fact, the very low estimates (<1) of the number of genes (Table 2) are indicative of monogenic inheritance. Due to the difficulty in assigning discrete phenotypic classes (resistant/susceptible) to some plants, frequency distributions of the numbers of galls and egg masses (Figures 1 and 2) in the parental (P_1 , P_2), F_1 , F_2 and backcross generations did not provide clear-cut evidence of monogenic inheritance. There was a range of gall and egg mass numbers that were common to both the resistant (cv. Carolina Cayenne) and the susceptible parent (cv. Agronômico-8).

Simulation models were used to test the hypothesis of monogenic inheritance. Based on the results of these simulations, within a range of degrees of dominance close to or only slightly above zero a hypothesis of monogenic inheritance cannot be ruled out (Figure 3). A single gene locus can therefore be presumed to control the resistance to *M. incognita* in cv.

Table 1. Estimates of additive ($\hat{\sigma}_A^2$), nonadditive ($\hat{\sigma}_D^2$) and environmental ($\hat{\sigma}_E^2$) variances, broad- and narrow-sense heritabilities and number of genes for number of galls and egg masses per plant.

	$\hat{\sigma}_A^2$ ± SEM	$\hat{\sigma}_D^2$ ± SEM	$\hat{\sigma}_E^2$	Heritability (broad sense)	Heritability (narrow sense)	Number of genes
Number of galls	46.26 ± 16.08	-11.73 ¹ ± 9.50	10.27	0.77 ± 0.12	0.77 ± 0.20	0.25
Number of egg masses	55.51 ± 34.13	8.35 ± 22.47	25.268	0.72 ± 0.13	0.63 ± 0.29	0.34

¹Negative estimate for which a true value of zero is assumed. SEM = standard error of the mean.

Table 2. Mean components for number of galls and of egg masses in the cross Agronômico-8 x Carolina Cayenne.

	Number of galls	Number of egg masses
m	8.128 ± 0.584	11.930 ± 0.552
a	4.431 ± 0.580	6.887 ± 0.551
d	1.279 ± 1.024	1.748 ± 0.913
χ^2	4.194 ^{ns}	1.427 ^{ns}
r ²	0.992	0.997
MDD	0.29	0.25

m = parental mean; a = additive mean component; d = nonadditive mean component; χ^2 = chi-square test; r² = coefficient of determination of the model; MDD = d/a = mean degree of dominance; ns = nonsignificant.

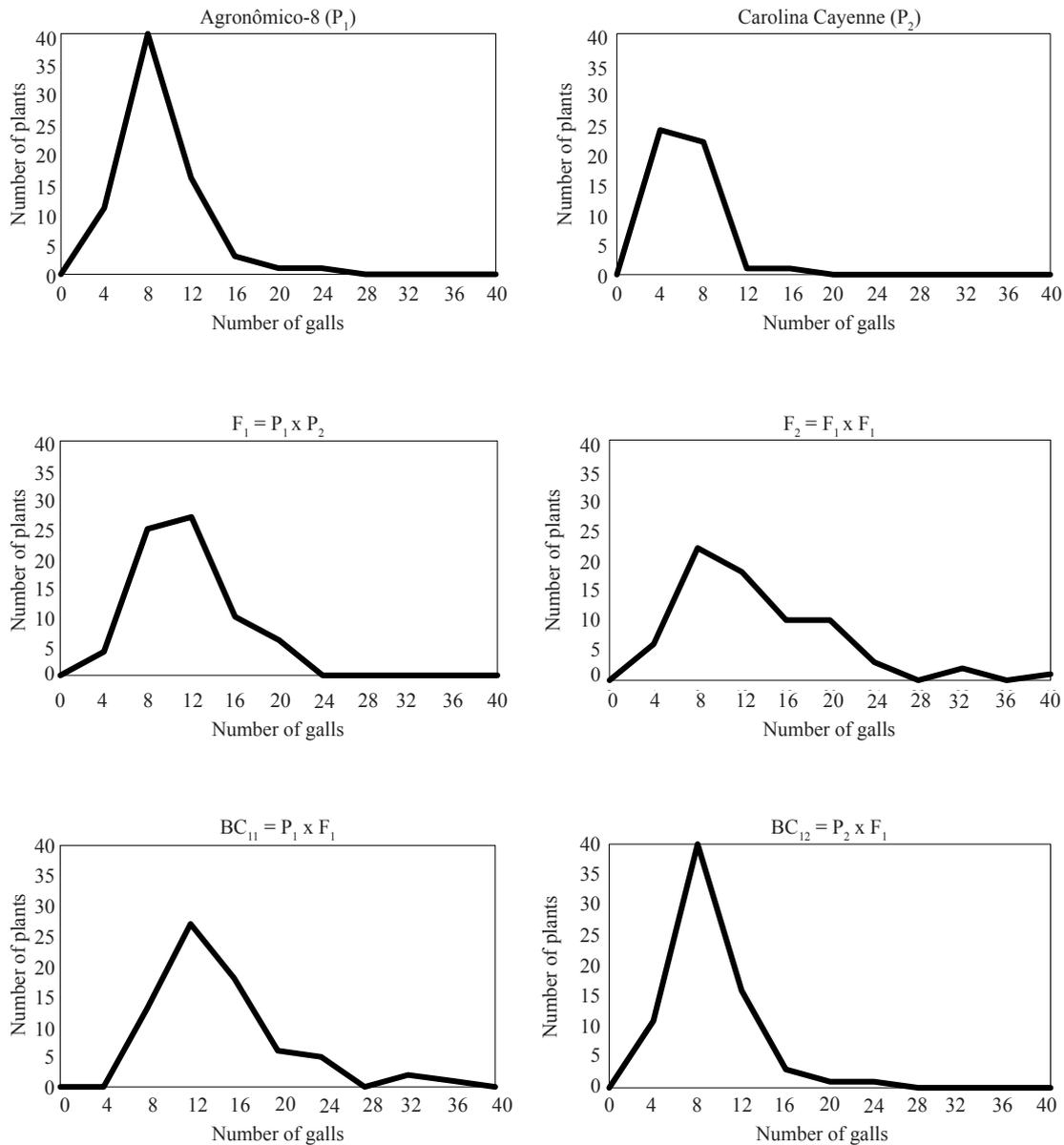


Figure 1. Frequency distribution of the number of galls in the parental lines Agronômico-8 (P_1), Carolina Cayenne (P_2), and generations F_1 ($P_1 \times P_2$), F_2 ($F_1 \times F_1$), and back crosses BC_{11} ($P_1 \times F_1$) and BC_{12} ($P_2 \times F_1$).

Carolina Cayenne. The point estimates of the MDD (Table 1) of 0.29 for galls and 0.25 for egg masses are situated within the range of values admissible for the acceptance of a monogenic hypothesis (Figure 3).

The evidence for monogenic control of nematode resistance in Carolina Cayenne contrasts with the results of Fery and Dukes (1996), who reported two genes, one dominant and one recessive, both of which controlled resistance in the Carolina Hot-derived line PA-135 (which is one of the component lines of cv. Carolina Cayenne). Different criteria for the evalu-

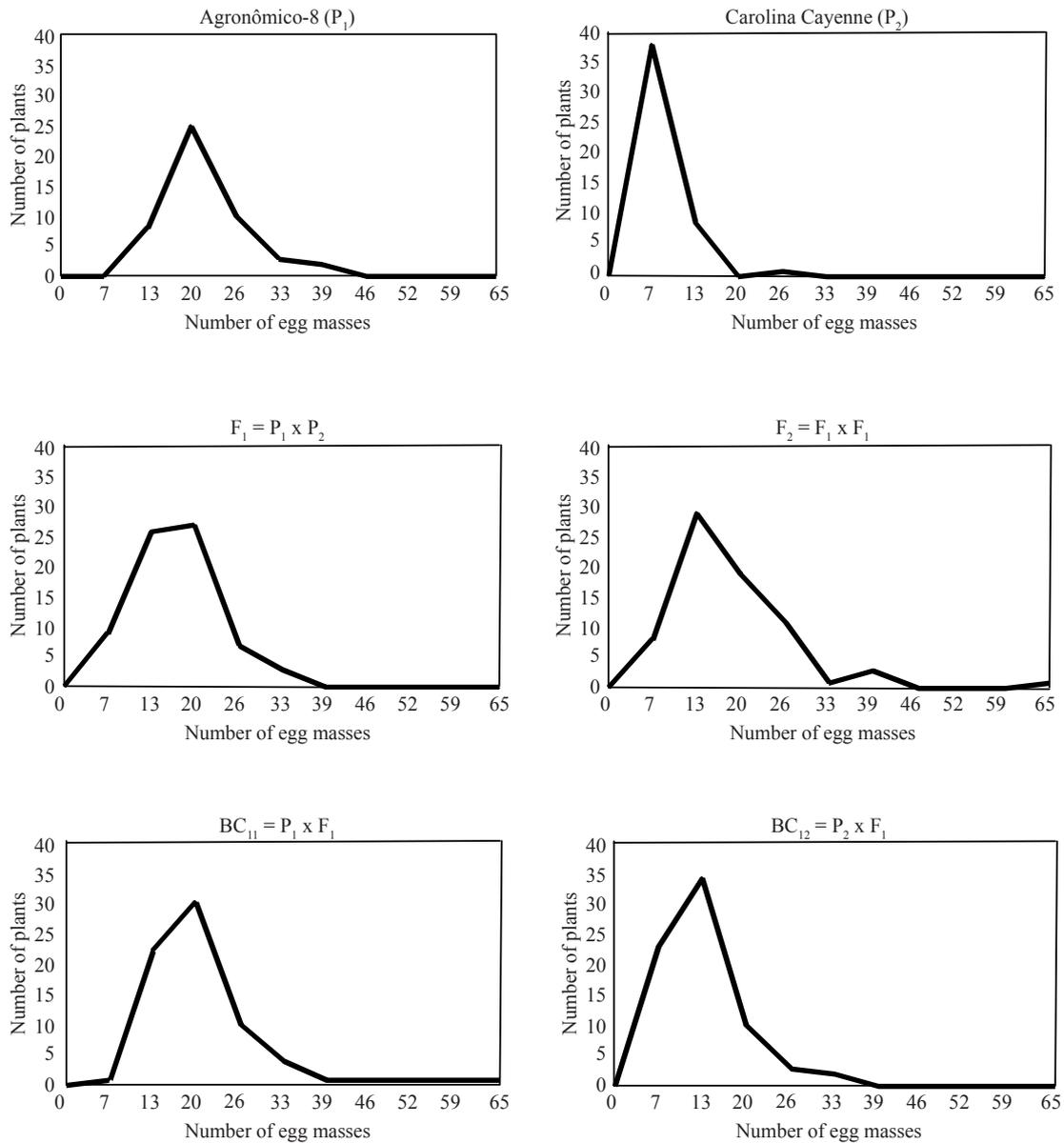


Figure 2. Frequency distribution of the number of egg masses in the parental lines Agronômico-8 (P_1), Carolina Cayenne (P_2), and generations F_1 ($P_1 \times P_2$), F_2 ($F_1 \times F_1$), and back crosses BC_{11} ($P_1 \times F_1$) and BC_{12} ($P_2 \times F_1$).

ation of resistance, different *M. incognita* races and/or differences in susceptible parent plants may account for the discrepancies between our results and those of Fery and Dukes (1996). The recessive gene described by Fery and Dukes (1996) would explain the differences in the degree of resistance between PA-135 and cv. Mississippi Nemaheart, both resistant to *M. incognita* and bearing the same dominant *N* gene described by Hare (1957). This recessive gene would account for the slightly higher degree of resistance found in PA-135, but would go undetected in our studies if it was ineffective against our *M. incognita* race 2 isolate or if it was also present

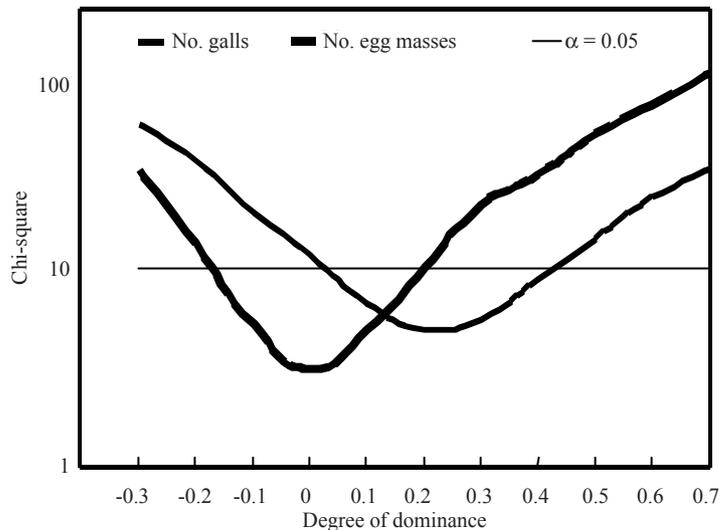


Figure 3. Chi-square values for the hypothesis of monogenic inheritance of gall and egg mass number under different presumed degrees of dominance.

in cv. Agrônômico-8. Some evidence supporting the presence of this recessive gene for resistance in cv. Agrônômico-8 is the slightly lower susceptibility of Agrônômico-8 compared to the susceptible lines Linha-004 and Linha-006 reported by Peixoto (1995).

As pointed out by Fery and Dukes (1996) the simple genetic control and the high heritability of *M. incognita* resistance in cv. Carolina Cayenne should favor breeding of resistant pepper inbred lines, but deployment of this source of resistance in hybrid breeding programs may be complicated by the additive gene action involved. High nematode resistance levels in hybrids can only be expected if both parental inbreds are resistant, a fact that may impose additional constraints on breeding programs.

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REFERENCES

- Di Vito, M. and Saccardo, F.** (1978). Risposta de linee e varietà di *Capsicum* agli attacchi di *Meloidogyne incognita* in serra. *Nematol. Mediterr.* 6: 83-88.
- Ferraz, S. and Mendes, M.L.** (1992). O nematóide das galhas. *Inf. Agropecu.* 16: 43-45.
- Fery, R.L. and Dukes, P.D.** (1996). The inheritance of resistance to the southern root-knot nematode in 'Carolina Hot' Cayenne pepper. *J. Am. Soc. Hort. Sci.* 121: 1024-1027.
- Hare, W.W.** (1956). Resistance in pepper to *Meloidogyne incognita acrita*. *Phytopathology* 46: 98-104.

- Hare, W.W.** (1957). Inheritance of resistance to root knot nematodes in pepper. *Phytopathology* 47: 455-459.
- Hendy, H., Pochard, E. and Dalmaso, A.** (1983). Identification de deux nouvelles souches de résistance aux nématodes du genre *Meloidogyne* chez le piment, *Capsicum annum* L. *C.R. Séances Acad. Agric. Fr.* 69: 817-822.
- Hendy, H., Pochard, E. and Dalmaso, A.** (1985). Transmission héréditaire de la résistance aux nématodes *Meloidogyne* Chitwood (Tylenchida) portée par 2 lignées de *Capsicum annum* L. Étude de descendance homozygotes issues d'androgenèse. *Agronomie* 5: 93-100.
- Hussey, R.S. and Barker, K.R.** (1973). A comparison of methods of selecting inocula of *Meloidogyne* spp. including a new technique. *Plant Dis. Rep.* 57: 1025-1028.
- Lordello, L.G.E.** (1984). *Nematóides das Plantas Cultivadas*. Nobel, São Paulo, SP, Brazil, pp. 314.
- Peixoto, J.R.** (1995). Melhoramento de pimentão (*Capsicum annum* L.) visando a resistência aos nematóides do gênero *Meloidogyne* spp. Doctoral thesis, Universidade Federal de Lavras, Lavras, MG, Brazil.
- Ramalho, M.A.P., Santos, J.B. and Pinto, C.A.B.P.** (1989). *Genética na Agropecuária*. Globo, São Paulo, SP, Brazil, pp. 359.
- Rowe, K.E. and Alexander, W.L.** (1980). Computations for estimating the genetic parameters in joint-scaling tests. *Crop Sci.* 20: 109-110.
- Taylor, A.L. and Sasser, J.N.** (1978). *Biology, Identification and Control of Root-Knot Nematodes (Meloidogyne species)*. University Graphics, Raleigh, NC, USA, pp. 111.
- Vencovsky, R. and Barriga, P.** (1992). *Genética Biométrica no Fitomelhoramento*. Revista Brasileira de Genética, Ribeirão Preto, SP, Brazil, pp. 486.
- Zamora, E., Bosland, P.W. and Thomas, S.** (1994). 'Carolina Cayenne' as a source of resistance to *Meloidogyne incognita* races 1, 2, 3 and 4. *HortScience* 29: 1184-1185.