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CROP PRODUCTION

Genetic parameters of resistance to *Meloidogyne incognita* in melon

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ABSTRACT: *In order to assess the genetic control of resistance in the melon 'Gaúcho Redondo' to the root-knot nematode Meloidogyne incognita, an experiment was conducted in a randomized complete block design with three blocks and six treatments using the parental lines 'Gaúcho Redondo' (P_1 resistant) and JAB 20 (P_2 susceptible), as well as F_1, F_2, and backcross generations (RC_1P_1 and RC_1P_2). Seventy days after inoculation, individual plants were evaluated for resistance using the nematode reproduction factor (RF). The hypothesis of monogenic inheritance was rejected by the chi-square test (χ^2), and results indicated that resistance is controlled by more than one gene locus, as confirmed by the quantitative analysis that revealed the presence of six genes.*
Key words: *Cucumis melo, genetic inheritance, plant breeding, reproduction factor.*

Parâmetros genéticos da resistência a *Meloidogyne incognita* em meloeiro

RESUMO: *Com o objetivo de avaliar o controle genético da resistência do melão 'Gaúcho Redondo' ao nematoide de galha Meloidogyne incognita, foi conduzido um experimento em blocos casualizados com três blocos e seis tratamentos, os quais envolveram as linhas parentais 'Gaúcho Redondo' (P_1, resistente) e JAB 20 (P_2, suscetível), assim como as gerações F_1, F_2, e retrocruzamentos (RC_1P_1 e RC_1P_2). Avaliaram-se plantas individuais após 70 dias da inoculação com o patógeno, por meio do fator de reprodução do nematoide (FR). A hipótese de herança monogênica foi rejeitada pelo teste do qui-quadrado (χ^2), indicando que a resistência está sob controle de mais de um loci gênico, sendo confirmado pela análise quantitativa, que evidenciou a presença de seis genes.*
Palavras-chave: *Cucumis melo, herança genética, melhoramento de plantas, fator de reprodução.*

INTRODUCTION

On a national scale, the melon (*Cucumis melo* L.) has been of great importance as a Brazilian export. Main destinations include the Netherlands, Spain, the United Kingdom, and Italy (AGRIANUAL, 2015). In addition, it has a significant social and economic impact, since its cultivation mainly requires qualified and intensive labor.

With an increase in production, the difficulty of cultivating this vegetable has also escalated, particularly due to the infestation of production areas by numerous phytopathogens, among which root-knot nematodes (*Meloidogyne* ssp.) cause the most severe damage. The disease is characterized by the destruction of root cells, chlorosis, and a reduction in leaf area and fruit quality, which results in decreased crop productivity (GALATTI et al., 2013).

The most important and frequent root-knot nematodes reported in Brazilian cucurbit crops are *Meloidogyne incognita* (Kofoid & White, 1919) Chitwood, 1949; *M. javanica* (Treub) Chitwood; and *M. arenaria* (Neal) Chitwood (PINHEIRO & AMARO, 2010).

According to PEIL (2003), the problems of soil infestation by root-knot nematodes are increasingly difficult to solve by traditional control methods. Therefore, the use of genetic resistance is an excellent form of control because it does not add to production costs nor does it contaminate the environment.

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Inheritance studies for this trait were already carried out in lettuce (*Lactuca sativa*) for races 1, 2, 3, and 4 of *M. incognita* (GOMES et al., 2000) and *M. javanica* (MALUF et al., 2002). These results showed that genetic control is exerted at a single gene locus and features a predominantly addictive effect, presenting relatively high broad-sense heritability.

Information regarding to the genetic control of traits linked to root-knot nematode resistance in melon is scarce in the literature. Such information is vital for decision-making about the appropriate breeding method and the size of the population to be assessed during the implementation of a breeding program aiming to obtain resistant lines.

The identification of resistance to *M. incognita* in the 'Gaúcho Redondo' cultivar, carried out by ITO et al. (2014), facilitated the study of inheritance for the trait in question. According to CRUZ & REGAZZI (1994), a genetic control study with generations F_1, F_2 and backcrosses, including parental P_1 and P_2, can lead to the quantification of the magnitude and nature of genetic variability in the segregating population and the assessment of the relative importance of genetic effects, which compose the averages of the studied populations.

Taking this into account, our study aimed to investigate the genetic control of melon resistance to *M. incognita* by estimating genetic parameters.

MATERIALS AND METHODS

The experiment was carried out in the Horticultural and Medicinal Plants Sector of the Department of Crop Production at São Paulo State University-UNESP/FCAV, Jaboticabal Campus.

Cross-breeding to obtain the F_1 hybrid, segregating generation (F_2) and backcross generations (RC_1P_1 and RC_1P_2).

The susceptible strain JAB 20 (*Cucumis melo* var. *cantalupensis* Naud.) was previously crossed with the 'Gaúcho Redondo' cultivar (*Cucumis melo* var. *reticulatus* Naud.), which is resistant to *M. incognita*, in order to obtain the F_1, RC_1P_1 and RC_1P_2, and F_2 populations.

The 'Gaúcho Redondo' cultivar was used as a female parent and the JAB 20 strain as a male parent. This strain was developed by the melon cross-breeding program of UNESP/FCAV and has high general combining ability (GCA), especially for mass (kg fruit^{-1}) and fruit production (kg m^{-2}) (VARGAS et al., 2010).

The seeds of the aforementioned genotypes were sown in trays of expanded polystyrene, with 128 cells each, and filled with Bioplant® substrate. Seedlings were individually transplanted to plastic pots with a capacity of 13L. Pots were previously filled with coconut husk fiber.

As soon as flowering began, artificial hybridization was carried out between progenitors, which differ in their resistance to *M. incognita*, and F_1 seeds were obtained. Backcross generations were obtained from crosses RC_1P_1 (F_1 x P_1) and RC_1P_2 (F_1 x P_2). In order to obtain the segregating generation (F_2), female flowers of F_1 plants were self-fertilized.

Inheritance of resistance

Once seeds from the generations involved in this study were obtained, the resistance test for *M. incognita* was carried out. A randomized block experimental design, with three repetitions, was adopted. Treatments were P_1 P_2, F_1, RC_1P_1, and RC_1P_2 and F_2. Experimental plots contained 10 plants for progenitors and F_1, 40 plants for backcross generations and 70 plants for F_2, for each block, respectively. Thus, the final population assessed of each generation consisted of 26 and 29 plants for progenitors P_1 and P_2, respectively, and 30, 209, 118, and 115 plants for F_1, F_2, RC_1P_1 and RC_1P_2 generations, respectively.

Several clay pots, with a capacity of 3L, were used, containing a mixture of coarse sand and soil as substrate, in a 3:1 ratio. The mixture was previously sieved and autoclaved at 120°C and 1atm, for 1 hour.

During transplantation, each plant was inoculated with 5.0mL of a suspension containing 3,000 eggs and second stage juveniles of *M. incognita* using an automated pipette. This was designated as the initial population (Pi). Eggs and second stage juveniles were extracted from cotton plants, and the species identifications were confirmed by examining morphological traits of the perineal pattern with an optical microscope.

In order to assess the viability and quality of the inoculum during nematode development, the same inoculum was used on tomato plants. Ten plants from a susceptible cultivar, 'Santa Cruz Kada', and ten plants from a resistant cultivar, 'AP-529', were used. The inoculum was prepared according to the technique described by HUSSEY & BARKER (1973), with the modifications introduced by BONETTI & FERRAZ (1981).

Assessment of plants inoculated with M. incognita

Seventy days after transplant and inoculation with nematodes, all inoculated plant

generations were analyzed. The nematode extraction was carried out according to the technique described by HUSSEY & BARKER (1973). The final population (Pf) of each suspension was used to determine the reproduction factor (RF), in which plants with a RF<1 were considered resistant, and plants with a FR≥1 were considered susceptible to the nematode, according to OOSTENBINK (1966).

Analysis of results

The data were subjected to a quantitative and qualitative analysis. For the qualitative analysis, plants from each generation were separated into resistant (R) or susceptible (S), according to the results of the RF values. A chi-square test (χ^2) was used to verify the hypothesis of dominant monogenic inheritance (3R:1S).

Quantitative genetic analyses were carried out using the software GENES, developed by the Universidade Federal de Viçosa (VFU) (CRUZ, 2013). According to CRUZ (2006), the following genetic parameters were estimated: Additive Variance ($\alpha_A^2 = 2\,\alpha_{F2}^2 - \alpha_{RC1P1}^2 - \alpha_{RC1P2}^2$), Dominant Variance ($\alpha_D^2 = \alpha_{F2}^2 - \alpha_A^2 - \alpha_E^2$), Environmental Variance ($\alpha_E^2 = \frac{\alpha_{P1}^2+\alpha_{P2}^2+2\alpha_{F1}^2}{4}$) and Genetic Variance ($\alpha_G^2 = \alpha_{F2}^2 - \alpha_E^2$), Broad-Sense Heritability ($h_a^2 = \frac{\alpha_G^2}{\alpha_{F2}^2}$) and Narrow-Sense Heritability ($h_r^2 = \frac{\alpha_A^2}{\alpha_{F2}^2}$), Average Dominance Index ($\frac{\sqrt{(2.\alpha_D^2}}{\sqrt{\alpha_A^2}}$), Gain by Selection ($\Delta G = Ds\ x$) and Number of Genes ($\eta = \frac{R^2(1+0.5.GMD^2)}{8\alpha_G^2}$).

RESULTS AND DISCUSSION

After the inoculation with the nematode *M. incognita*, all parental plants P_1 were resistant, while parental plants P_2 were susceptible (Table 1). According to the results of the chi-square test with a 1% level of significance, it was possible to reject the hypothesis of monogenic inheritance, as results indicated that more genes are involved in the control of resistance.

Based on the quantitative analysis, the inheritance of the resistance trait to *M. incognita* may be defined as polygenic, with an estimated involvement of 6.07 genes (Table 2). Results of our study differ from those obtained by CARVALHO FILHO et al. (2008) and SOBRINHO-SOUZA et al. (2002) who examined the genetic control of resistance of lettuce 'Salinas 88' to *M. incognita* race 1 and pepper 'Carolina Cayenne' to *M. incognita* race 2, respectively. They verified the presence of only one major gene with additive effects regulating resistance, and thereby concluded that, depending on the culture studied, the genetic control of resistance to *M. incognita* might be regulated by several different genes.

FERREIRA et al. (2010) studied the resistance of cultivars of *Phaseolus vulgaris* L. to *M. incognita* (races 1 and 3) and *M. javanica*. Their results showed that resistance might be controlled by different genes, depending on the species and race of *Meloidogyne* considered in the study, thus confirming that a different genetic control may be observed in different cultures, as reported in our study with melon 'Gaúcho Redondo'.

Transgressive segregation was observed for the F_2 generation, both for susceptibility and resistance, with some plants showing a maximum RF of 27.87, and others a minimum RF of 0.08. This shows average RF values outside the upper and lower resistance limits of the progenitors, suggesting the occurrence of an additional gene involved in the determination of resistance or presence of epistasis. In their study of inheritance of resistance to potyvirus Pepper yellow mosaic virus (PepYMV) in tomato plants, JUHÁSZ et al. (2008) explained transgressive segregation in the F_2 generation by the presence of an additional gene involved in the determination of resistance.

In our study, broad-sense and narrow-sense heritability were 76.91% and 72.17%, respectively, showing that the resistance trait to *M. incognita* is determined to a greater extent by additive gene action; and therefore, it responds favorably to the selection based on individuals with lower RF values (Table 2). Similar results were obtained by CARVALHO FILHO et al. (2011), who studied population parameters and correlations among characteristics of resistance to *M. incognita* in lettuce. They obtained a 72% broad-sense heritability for the number of eggs, showing the possibility for success in the selection of plants resistant to *M. incognita* race 1. Regarding the root-knot index, CARVALHO FILHO et al. (2012) obtained a 74% broad-sense heritability in progenies of lettuce $F_{2:3}$ derived from a cross between 'Salinas 88' and 'Colorado'.

The average dominance index obtained in this study was 0.36, showing an allelic interaction of

Table 1 - Reaction of melon plants inoculated with *Meloidogyne incognita* assessed by the reproduction factor (RF), considering the number of eggs + juveniles (J2). UNESP/FCAV, Jaboticabal-SP, 2013.

Generations	Number Of Plants	RF[(1)] Average	Observed Proportions R/S[(2)]	Variance	Standard-Deviation
P_1[(a)]	26	0.68	26/0	0.44	0.66
P_2[(b)]	29	4.93	0/29	10.73	3.28
F_1	30	3.66	2/28	4.79	2.19
F_2	209	2.97	80/129	22.04	4.69
RC_1P_1	118	2.81	28/90	6.42	2.53
RC_1P_2	115	4.49	12/103	21.74	4.66

[(1)]RF Values ≥1.0 indicate susceptibility and RF<1.0 indicate resistance to *Meloidogyne incognita*.
[(2)]Frequency of resistant (R) (RF<1.0) and susceptible plants (S) (FR≥1.0) in the analyzed generations.
[(a)]Melon 'Gaúcho Redondo' (resistant to the nematode).
[(b)]Melon strain JAB 20 (susceptible to the nematode).

incomplete dominance (Table 2). Similar results were obtained by FERREIRA et al. (2012) in a study of genetic control of resistance to *M. incognita* race 1 in *Phaseolus vulgaris* L.; incomplete dominance of the allele that controls resistance was observed.

Genotypic variance observed in the F_2 generation in this study might be attributed primarily to additive genetic effects, since the variance estimates of this effect were substantially greater than the variance estimates of dominance (Table 2). Furthermore, it was possible to observe that the genetic gain predicted by selection in the F_2 generation, based on plants resistant to *M. incognita*, was -63.99% (Table 2). Negative value indicated that selection occurs for plants with lower RF values.

Genetic effects observed in our study, specifically those involved in the inheritance of the resistance to nematodes, may be explained to a large extent by the additive-dominant model, represented by the sum of the effects of the mean (m), additive gene action (a), and dominance (d), since the correlation between the averages estimated by the model and the averages obtained from the generations was high ($R^2 = 0.9745$). In their study of bean plants with resistance to *M. incognita* race 1, FERREIRA et al. (2012) also verified that the additive-dominant model was sufficient to explain the data, since the correlation obtained was also high ($R^2 = 0.9881$).

In the additive-dominant model, the estimate of the additive genetic effect was greater than that of dominance (a = 33.96% and d = 5.29%) (Table 3). Thus, the additive genetic portion involved in the trait studied is significant; it positively responds to the phenotype-based selection of segregating generations.

The complete model, represented by the sum of effects of the mean (m), additive (a), dominance (d), and their interactions (aa, ad, dd),

Table 2 - Estimates of the genetic parameters obtained through variance analyses for resistance to *Meloidogyne incognita* in the F_2 population. UNESP/FCAV, Jaboticabal-SP, 2013.

Genetic parameters	Estimates
Phenotypic variance	22.04
Environmental variance	5.09
Genotypic variance	16.95
Additive variance	15.90
Dominance variance	1.05
Broad-Sense heritability (%)	76.91
Narrow-Sense heritability (%)	72.17
Average dominance index	0.36
Number of genes	6.07
Gain by selection (%)	-63.99

Table 3 - Non-orthogonal decomposition of the square sum (SS) and determination coefficient (R^2) of the parameters (m, a, d) for the additive-dominant model and parameters (m, a, d, aa, ad, dd) for the complete model, based on the averages of the reproduction factor (RF) of six generations (P_1, P_2, F_1, F_2, RC_1P_1 and RC_1P_2), in populations of *Cucumis melo* inoculated with *Meloidogyne incognita*. UNESP/FCAV, Jaboticabal-SP, 2013.

Source of variation	SQ	R^2 (%)
m/a,d	120.20	60.75
a/m,d	67.20	33.96
d/m,a	10.47	5.29
Total	197.87	100%
m/a,d,aa,ad,dd	0.03	0.00
a/m,d,aa,ad,dd	48.75	82.81
d/m,a,aa,ad,dd	3.82	6.50
Total	52.60	89.31
aa/m,a,d,ad,dd	2.75	4.67
ad/m,a,d,aa,dd	0.60	1.03
dd/m,a,d,aa,ad	2.92	4.96
Total of epistasis	6.27	10.69

m = mean effect; a = additive effect; d = dominance deviation effect; aa = additive-additive effect; ad = additive-dominant effect; dd = dominant-dominant effect.

showed that the effect of epistasis contributed 10.69% (R^2) of the total variability, indicating that the epistatic effects were not important in the genetic control of the analyzed trait (Table 3).

Because the inheritance of resistance is controlled by 6.07 genes, the method of backcross generations, widely used to transfer resistance genes in plants, would not be appropriate in this particular case of resistance. The transfer of resistance genes would require the development of more complex selection strategies, such as a recurrent selection.

CONCLUSION

An analysis of generations is effective in determining the number of genes involved in the inheritance of resistance to *M. incognita*. In *C. melo*, the genetic control of resistance to *M. incognita* is polygenic and ruled by six gene loci, with incomplete dominance of the resistance allele and the presence of additive genetic effects.

REFERENCES

AGRIANUAL. **Anuário da agricultura brasileira**. 20.ed. São Paulo: AgraFNT. 2015. p. 480.

CARVALHO FILHO, J.L.S. et al. Incidence of the root-knot *Meloidogyne incognita* race 1 in $F_{2:3}$ ('Salinas 88' x 'Colorado') progenies of lettuce. **Scientia Plena**, v.8, n.2, p.01-07, Feb. 2012. Available from: <http://www.scientiaplena.org.br/sp/article/view/251/438>. Accessed: June 20, 2015.

CARVALHO FILHO, J.L.S. et al. Population parameters and correlation between resistance trait to root-knot nematodes in lettuce. **Revista Brasileira de Ciências Agrárias**, v.6, n.1, p.46-51, Jan-Mar. 2011. Available from: <http://dx.doi.org/10.5039/agraria.v6i1a819>. Accessed: Jan. 10, 2014. doi: 10.5039/agraria.v6i1a819.

CARVALHO FILHO, J.L.S. et al. Inheritance of resistance of 'Salinas 88' lettuce to the root-knot nematode *Meloidogyne incognita* (Kofoid & White). **Revista Brasileira Agrociência**, v.14, n.2, p.279-289, Apr-Jun. 2008. Available from: <http://periodicos.ufpel.edu.br/ojs2/index.php/CAST/article/view/1912/1745>. Accessed: Jan. 10, 2014.

CRUZ, C.D. GENES - A software package for analysis in experimental statistics and quantitative genetics. **Acta Scientiarum Agronomy**, v.35, n.3, p.271-276, Jul-Sept. 2013. Available from: <http://periodicos.uem.br/ojs/index.php/ActaSciAgron/article/view/21251>. Accessed: Jan. 20, 2014. doi: 10.4025/actasciagron.v35i3.21251.

CRUZ, C.D.; REGAZZI, A.J. **Modelos biométricos aplicados ao melhoramento genético**. Vicosa: Editora UFV, 1994. 668p.

CRUZ, C.D. **Programa GENES**: biometria. Viçosa: UFV, 2006. 382p.

FERREIRA, S. et al. Resistance of dry bean and snap bean cultivars to root-knot nematodes. **HortScience**, v.45, n.2, p.320-322, Feb. 2010. Available from: <http://hortsci.ashspublications.org/content/45/2/320.full.pdf+html>. Accessed: Jan. 22, 2014.

FERREIRA, S. et al. Genetic control of resistance to *Meloidogyne incognita* race 1 in the Brazilian common bean (*Phaseolus vulgaris* L.) cv. 'Apore'. **Euphytica**, v.186, n.3, p.867-873, Aug. 2012. Available from: <http://dx.doi.org/10.1007/s10681-012-0655-7>. Accessed: Jan. 20, 2014. doi: 10.1007/s10681-012-0655-7.

GALATTI, F.S. et al. Rootstocks resistant to *Meloidogyne incognita* and compatibility of grafting in net melon. **Revista Ceres**, v.60, n.3, p.432-436, May-Jun. 2013. Available from: <http://dx.doi.

org/10.1590/S0034-737X2013000300018>. Accessed: Jan. 10, 2014. doi: 10.1590/S0034-737X2013000300018.

GOMES, L.A.A. et al. Inheritance of the resistant reaction of the lettuce cultivar 'Grand Rapids' to the southern root-knot nematode ***Meloidogyne incognita*** (Kofoid & White) Chitwood. **Euphytica**, v.114, n.1, p.37-46, July. 2000. Available from: <http://link.springer.com/article/10.1023%2FA%3A1003991819523>. Accessed: Jan. 20, 2014.

HUSSEY, R.S.; BARKER, K.R. A comparison of methods of collecting inocula of *Meloidogyne* spp., including a new technique. **Plant Disease Report**, v.57, p.1.025-1.028, Jul-Dec. 1973.

ITO, L.A. et al. Resistance of Cucurbita rootstocks to nematodes and compatibility of grafting with net melon. **Horticultura Brasileira**, v.32, n.3, p. 97-302, July-Sept. 2014. Available from: <http://www.scielo.br/scielo.php?script=sci_arttext&pid=S0102-05362014000300297>. Accessed: June 23, 2015. doi: 10.1590/S0102-05362014000300010.

JUHÁSZ, A.C.P. et al. Genetic basis of resistance in wild tomato to the pepper yellow mosaic virus. **Pesquisa Agropecuária Brasileira**, v.43, n.6, p.713-720, June, 2008. Available from: <http://www.scielo.br/scielo.php?pid=S0100-204X2008000600007&script=sci_abstract&tlng=pt>. Accessed: June 25, 2015. doi: 10.1590/S0100-204X2008000600007.

MALUF, W.R. et al. Inheritance of resistance to the root-knot nematode *Meloidogyne javanica* in lettuce. **Genetics and Molecular Research**, v.1, n.1, p.64-71, Mar. 2002. Available from: <http://www.funpecrp.com.br/gmr/year2002/vol1-1/gmr0008_abstract.htm>. Accessed: June 18, 2015.

OOSTENBRINK, M. Major characteristics of the relation between nematodes and plants. **Mededelingen Landbouwhogeschool**, v.66, p.1-46, 1966. Available from: <http://nematologia.com.br/wp-content/uploads/2012/07/most66.pdf>. Accessed: Jan. 20, 2014.

PEIL, R.M. Grafting of vegetable crops. **Ciência Rural**, v.33, n.6, p.1169-1177, Nov-Dec. 2003. Available from: <http://www.scielo.br/pdf/cr/v33n6/a28v33n6.pdf>. Accessed: Jan. 20, 2014.

PINHEIRO, J.B.; AMARO, G.B. **Ocorrência e controle de nematoides nas principais espécies cultivadas de Cucurbitáceas**. Brasília-DF: Embrapa Hortaliças, 2010. 7p. Circular Técnica, 88.

SOBRINHO-SOUZA, F. et al. Inheritance of resistance to ***Meloidogyne incognita*** race 2 in the hot pepper cultivar 'Carolina Cayenne' *Capsicum annuum* (L.). **Genetics and Molecular Research**, v.1, n.3, p.271-279, Sept. 2002. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/14963835>. Accessed: Jan. 20, 2014.

VARGAS, P.F. et al. Performance of parents and experimental hybrids of net melon. **Ciência e Agrotecnologia**, v.34, n.5, p.1102-1108, Sept-Oct. 2010. Available from: <http://www.scielo.br/scielo.php?script=sci_arttext&pid=S1413-70542010000500004>. Accessed: June 25, 2015. doi: 10.1590/S1413-70542010000500004.