









Reaction of tomato germplasm accessions to root-knot nematode (*Meloidogyne incognita*)

José Carlos Lambert¹ Antonio Fernando de Souza¹ Talles de Oliveira Santos²
Rafael Nunes de Almeida³ Aparecida de Fátima Madella de Oliveira⁴
José Dias de Souza Neto⁴ Alexandre Cristiano Santos Júnior⁴ Monique Moreira Moulin^{4*}

¹Laboratório de Diagnóstico de Doenças de Plantas, Instituto Federal do Espírito Santo (IFES), Santa Teresa, ES, Brasil.

²Laboratório de Genética e Melhoramento de Plantas, Universidade Estadual do Norte Fluminense (UENF), Campos dos Goytacazes, RJ, Brasil.

³Centro de Pesquisa e Desenvolvimento em Inovação Norte, Instituto Capixaba de Pesquisa, Assistência Técnica e Extensão Rural (Incaper), Linhares, ES, Brasil.

⁴Laboratório de Genética e Biologia Molecular, Instituto Federal do Espírito Santo (IFES), 29500-000, Alegre, ES, Brasil. E-mail: moniquemoulin@gmail.com. *Corresponding author.

ABSTRACT: Tomato is one of the most produced and consumed vegetables in the world. Among the range of diseases affecting tomato plants are the root-knot nematodes of the genus *Meloidogyne*, which cause significant annual production losses. In Brazil, species of the genus *Meloidogyne* are the main culprits of damage to tomato crops, with *Meloidogyne incognita* being the most prevalent. Its control is quite challenging, as chemical control is not very effective, costly, and poses risks of environmental and human contamination. The most economical and effective alternative is the use of resistant genotypes selected through breeding programs. This study evaluated the tomato accessions for resistance to *M. incognita*. A total of 43 accessions were evaluated, of which 40 belonged to the Germplasm Bank of Ifes Campus of Alegre, along with two commercial cultivars, 'BRS Portinari' and 'Grande HT', which are resistant to *Meloidogyne incognita* and were used as controls, as well as the 'Santa Clara' cultivar, which is susceptible to *Meloidogyne* spp. and was used as a reference. Two experiments were conducted in a shaded nursery using a randomized block design with five replications and six plants as experimental unit. Four thousand eggs + J2 of *M. incognita* were inoculated per pot, and 65 days after planting, the genotypes were evaluated. The extraction and counting of nematode eggs were performed. The classification of resistance levels was conducted using the reproduction factor (FR) and reproduction index (IR). It was found that eight accessions (Ifes 5, 14, 23, 26, 27, 33, 44, and 45) exhibited a reproduction factor less than one ($FR < 1$), classifying them as resistant in both experiments, consistent with the results obtained for the reproduction index. A consistent response was observed among the 43 evaluated genotypes regarding resistance and susceptibility in both experiments. According to the two classifications used, six accessions and the two commercial cultivars used as controls were considered promising for further study and use as sources of resistance to *M. incognita*, as well as in targeted crosses in breeding programs.

Key words: *Meloidogyne* spp., root-knot nematodes, reproduction factor, reproduction index.

Reação de acessos de germoplasma de tomate ao nematoide-das-galhas (*Meloidogyne incognita*)

RESUMO: O tomate (*Solanum lycopersicum* L.) é uma das hortaliças mais produzidas e consumidas no mundo. Dentre a gama de doenças que afetam o tomateiro, estão os nematoides formadores de galhas, do gênero *Meloidogyne*, que causam grandes perdas na produção anualmente. No Brasil, espécies do gênero *Meloidogyne* são as principais causadoras de danos ao tomateiro, sendo *Meloidogyne incognita* o mais incidente. Seu controle é bastante difícil, sendo o controle químico pouco eficiente, oneroso, além do risco de contaminação do ambiente e do homem. A alternativa mais econômica e eficaz é a utilização de genótipos resistentes selecionados por programas de melhoramento genético. O objetivo deste trabalho foi avaliar acessos de tomate quanto à resistência à *M. incognita*. Avaliaram-se 43 acessos, sendo 40 destes pertencentes ao Banco de Germoplasma do Ifes Campus de Alegre, duas cultivares comerciais 'BRS Portinari' e 'Grande HT', resistentes ao *Meloidogyne incognita* e utilizadas como testemunhas, além do tomateiro cultivar 'Santa Clara', suscetível ao *Meloidogyne* spp. e utilizada como controle. Foram conduzidos dois experimentos em um viveiro telado e o delineamento experimental utilizado foi de blocos casualizados com cinco repetições e seis plantas por parcela. Foram inoculados 4.000 ovos + J2 de *M. incognita* por vaso e 65 dias após o cultivo os genótipos foram avaliados. Foi realizada a extração dos ovos dos nematoides e procedeu-se à sua contagem. A classificação dos níveis de resistência foi realizada pelo fator de reprodução (FR) e pelo índice de reprodução (IR). Verificou-se que oito acessos (Ifes 5, 14, 23, 26, 27, 33, 44 e 45) apresentaram fator de reprodução menor que um ($FR < 1$) sendo classificados como resistentes nos dois ensaios, estando em consonância com os resultados obtidos para o índice de reprodução. Foi possível constatar uma constância na resposta dos 43 genótipos avaliados quanto à resistência e suscetibilidade nos dois ensaios. De acordo com as duas classificações utilizadas, seis acessos e as duas cultivares comerciais utilizadas como testemunhas foram consideradas como promissoras para serem estudadas e utilizadas como fonte de resistência ao *M. incognita* e em cruzamentos direcionados em programas de melhoramento genético.

Palavras-chave: *Meloidogyne* spp., nematoides-das-galhas, fator de reprodução, índice de reprodução.

INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is one of the main vegetables cultivated worldwide due to its easy adaptation to various soil types and climates, reaching the market in both fresh and processed forms (ARAÚJO et al., 2018). It is the second most cultivated horticultural product in the world, after the potato, and the first in terms of industrial volume (CONAB, 2019). Global tomato production reached 182 million tons in 2022, with a production value exceeding 20 trillion tons (FAO, 2024). Besides its high economic value, tomatoes contain abundant metabolites such as soluble solids, flavonoids, polyphenols, hydroxycinnamic acids, vitamins, and minerals, providing a variety of essential nutrients required by humans (TIEMAN et al., 2017; GARBOWICZ et al., 2018).

Cultivated tomatoes have about 12 wild species that have a great genetic diversity, which has greatly contributed to the creation of modern tomato cultivars (CAMPOS et al., 2021). However, cultivated tomatoes have undergone severe genetic erosion during domestication, resulting in an extremely narrow genetic base that represents only 4.5% of the total genetic variation among tomato species (ASHRAFI et al., 2009; WANG et al., 2024). The replacement of local varieties by commercial cultivars has led to a continuous process of genetic erosion (DE PAULA et al., 2024). FERNANDES et al. (2018) and CAMPOS et al. (2021) emphasized the importance of rescuing traditional tomato materials from rural producers, as well as performing crossbreeding to expand the genetic base of the crop.

Brazil is the fifth-largest producer of processing tomatoes in the world, and its cultivation is a significant activity for generating employment, with a high demand for labor from the pre-planting phase to commercialization (TREICHEL et al., 2016). The tomato production system in Brazil has faced some limitations regarding technical standards due to the use of cultivars susceptible to pests and diseases, which leads to the excessive use of pesticides, posing risks to human health and the environment (BALBINO et al., 2014).

Among the range of pathogens that affect tomato plants are nematodes of the genus *Meloidogyne*, known as root-knot nematodes. These organisms infect the root system, forming galls that impede water and nutrient absorption, leading to significant losses in agricultural production and creating entry points for other pathogens (ÁVILA JÚNIOR, 2017). They are sedentary endoparasitic

phytoparasites that spend part of their life cycle in the soil, spreading mainly through water, infested seedlings, agricultural implements, and machinery etc. (CHARCHAR & LOPES, 2005).

The genus *Meloidogyne* comprises approximately 100 species (PHILBRICK et al., 2020). Various species of *Meloidogyne* have been found in production fields, including *M. arenaria*, *M. hapla*, *M. enterolobii*, *M. incognita*, and *M. javanica* (SILVA et al., 2019). In Brazil, the most important species in tomato cultivation are *Meloidogyne incognita* and *Meloidogyne javanica* (CHARCHAR & LOPES, 2005). *Meloidogyne incognita* is the root-knot nematode that causes the most qualitative losses to the tomato crop (JATALA, 1990). The main symptoms caused in the plant are galls, cracks, and reduction of the root system, decreased vigor, stunted growth, leaf yellowing, nutritional deficiencies, plant wilting – especially during the hottest hours of the day – and in cases of severe attacks, premature plant death (EL-SAPPAH et al., 2022).

Genetic resistance is the most efficient and sustainable strategy for controlling *Meloidogyne* spp. in tomatoes (PADILLA-HURTADO et al., 2022). Breeding for nematode resistance has been researched for over 60 years, based on a dominant gene (designated *Mi* 1-2), sourced from the wild tomato *Solanum peruvianum* (SILVA et al., 2019). In this context, characterizing germplasm banks to find sources of nematode resistance is of great relevance for tomato cultivation and the agroecological cultivation system. Significant efforts have been made to find new sources of resistance or tolerance to *M. incognita* in tomatoes.

Although, some commercial cultivars show resistance to *M. incognita* due to the presence of the *Mi* gene, it is important to note that because of emergence of new races of the pathogen this resistance is constantly being broken, highlighting the importance of breeding programs in seeking new sources of resistance to *Meloidogyne* spp. Therefore, this study evaluated the resistance of tomato accessions to the nematode *M. incognita*, using the classification criteria of OOSTENBRINK (1966) and TAYLOR (1967).

MATERIALS AND METHODS

The experiment was conducted in the shaded nursery of the Experimental Station for Vegetable Crops, and evaluations were performed in the Plant Disease Diagnosis Laboratory (LDDP), both located at the Instituto Federal do Espírito

Santo (Ifes) – Campus Santa Teresa, in Santa Teresa, Espírito Santo, Brazil (19°48'50" S, 40°40'44" W, 137m above sea level).

Two trials (named Experiment 1 and Experiment 2, for future reference) were carried out from March to June and from August to November of 2022. A total of 43 tomato accessions from the Ifes Active Germplasm Bank were characterized, comprising 40 traditional accessions from family farming, cultivated for decades by rural producers from the Central Serrana, Southwest Serrana, and Caparaó microregions of Espírito Santo; and two commercial cultivars ('BRS Portinari' and 'Grande HT'), used as resistant controls against *Meloidogyne incognita* (accessions Ifes 42 and Ifes 44), and the cultivar 'Santa Clara' (Ifes 45), which is susceptible to *Meloidogyne* spp., used as a control (Table 1).

Seedlings were produced by sowing seeds in 72-cell polystyrene trays, each filled with approximately 120 mL of commercial substrate (Plantmax®). After 20 days of growth, the seedlings were transplanted into individual pots for the experiment. Plants were grown in 2.8 L pots filled with a soil-based substrate composed of Red Latosol and sand in a 3:1 ratio. Each experimental unit consisted of six individually potted plants. The experiment was arranged in blocks, each comprising 138 plants (6 plants per unit × 43 genotypes/treatments). Pots were spaced 0.4 m apart within rows and 0.8 m between rows. Each block covered an area of 86.4 m². Experiment 1 included five replicates (432 m² total), while Experiment 2 included three replicates (259.2 m² total).

To infest the substrate, eggs were previously extracted from highly infected 'Santa Clara' tomato plants maintained in a greenhouse and susceptible to *M. incognita* populations. The inoculum was obtained using the methodology described by HUSSEY & BARKER (1973), modified by BONETTI & FERRAZ (1981). For quantification, the eggs collected through the finest sieve were transferred with the aid of a pipette with water into a beaker, and the volume was adjusted to 1 L. Three aliquots of 1 mL of the suspension were transferred to a Peters chamber and examined under a stereomicroscope to determine the average number of eggs per mL, followed by calibration of the suspension to the desired concentration. To confirm the species as *M. incognita*, root samples from inoculated tomato plants containing galls of *Meloidogyne* spp. were sent to the Nematology and Soil Microbiology Laboratory of the Instituto Capixaba de Pesquisa, Assistência Técnica e Extensão Rural (Incaper – ES), using the standard isoenzyme electrophoresis technique for

diagnosing *Meloidogyne* species (ESBENSHADE & TRIANTAPHYLOU, 1990).

Eight days after transplanting, each plant received 2 mL of the suspension containing 4,000 eggs + J2 through three small holes in the substrate around the plant's collar using an automatic syringe. In an incubation chamber, the viability of the inoculum was assessed, yielding 59% viable eggs, which corresponds to 2,360 viable *M. incognita* eggs applied per plant.

Sixty-five days after the substrate infestation, a careful evaluation of the plants was conducted, discarding the plant's shoot. The root system of each plant was washed by submersion in running water; and subsequently, the roots were processed according to the methodology proposed by HUSSEY & BARKER (1973), modified by BONETTI & FERRAZ (1981). The root system of each treatment was chopped, weighed, crushed, sieved, and processed using the centrifugal flotation method (JENKINS, 1964). The suspension was collected in a test tube for verification of the presence of eggs and juveniles of *M. incognita*.

Subsequently, the total number of eggs was quantified by extrapolating the count from 1 mL of the suspension in a Peters chamber under a stereomicroscope. This procedure yielded the final population (PF) of nematodes in the tomato plants. The variables Reproduction Factor (FR) and Reproduction Index (IR) were utilized to assess the resistance of the tomato accessions to *M. incognita*.

The reproduction factor was determined by the formula: $FR = PF/PI$, where FR: reproduction factor; PF: final population; and PI: initial population (number of eggs used in inoculations). The criteria for designating the host level of each genotype in the two trials were established according to OOSTENBRINK (1966), with tomato accessions having $FR \geq 1.00$ considered susceptible and those with $FR < 1.00$ considered resistant.

The calculation of the reproduction index (IR) was performed by considering the 'Santa Clara' tomato as the standard of susceptibility (100%) in relation to the reproduction of nematodes obtained in the other tomato accessions. Thus, the values obtained for the final population (PF) of tomato were divided by the final population found in the 'Santa Clara' tomato. The resulting values from the calculation for the reproduction index allowed for the classification of tomato accessions according to the classification established by TAYLOR (1967), where: S – susceptible plant, normal reproduction, IR above 51%; LR – slightly resistant, IR from 26 to 50%;

Table 1 - Passport information (identification, common name, and origin) of the 43 tomato accessions belonging to the Active Germplasm Bank of Ifes – Campus de Alegre.

Accession ID	Common name	Origin
Ifes 01	Tomate	Venda Nova do Imigrante
Ifes 02	Tomate	Santa Teresa
Ifes 03	Italiano	Venda Nova do Imigrante
Ifes 04	Cereja	Guaçuí
Ifes 05	Tomate	Guaçuí
Ifes 06	Tomate	Guaçuí
Ifes 07	Cereja	Guaçuí
Ifes 08	Tomate	Guaçuí
Ifes 09	Tomatão	Santa Leopoldina
Ifes 10	Italiano	Santa Leopoldina
Ifes 11	Tomate	Santa Leopoldina
Ifes 12	Tomate	Venda Nova do Imigrante
Ifes 13	Italiano	Venda Nova do Imigrante
Ifes 14	Cereja	Conceição de Castelo
Ifes 15	Tomate	Afonso Cláudio
Ifes 16	Tomate	Alegre
Ifes 17	Tomate	Alegre
Ifes 18	Cereja grande	Muniz Freire
Ifes 20	Batalha	Venda Nova do Imigrante
Ifes 21	Fusio	Venda Nova do Imigrante
Ifes 22	Maragadú	Venda Nova do Imigrante
Ifes 23	Batalha	Venda Nova do Imigrante
Ifes 24	TMINO	Venda Nova do Imigrante
Ifes 26	Teste 2	Venda Nova do Imigrante
Ifes 27	Teste 3	Venda Nova do Imigrante
Ifes 28	Sufia	Venda Nova do Imigrante
Ifes 29	Fusio	Venda Nova do Imigrante
Ifes 30	Italiano	Venda Nova do Imigrante
Ifes 31	Parão I	Venda Nova do Imigrante
Ifes 32	Parão II	Venda Nova do Imigrante
Ifes 33	Parão III	Venda Nova do Imigrante
Ifes 34	Italiano	Venda Nova do Imigrante
Ifes 35	TLV	Venda Nova do Imigrante
Ifes 36	Tomate	Domingos Martins
Ifes 37	Tomate	Domingos Martins
Ifes 38	Tomate	Santa Maria de Jetibá
Ifes 39	Baiano	Santa Teresa
Ifes 40	Português	Santa Teresa
Ifes 41	Holandês	Santa Teresa
Ifes 42	Tomatão	Santa Teresa
Ifes 43	‘Santa Clara’	Comercial
Ifes 44	‘Grande HT’	Comercial
Ifes 45	‘BRS Portinari’	Comercial

MoR – moderately resistant, with IR from 11 to 25%; MR – very resistant, IR from 1 to 10%; AR/I – highly resistant/immune, IR below 1%.

To meet the assumptions of the statistical analysis, the values obtained from the resistance

classifications used, derived from counts, were subjected to a log transformation ($x + 2$); however, the original values are presented in the tables. The data were subjected to analysis of variance, and when significant differences were identified by the F test,

they were grouped using the Scott-Knott test at a 5% probability level.

Broad-sense heritability ($h^2 = V_g/V_e$) was also estimated, along with the genetic variation coefficient (calculated as $CV_g(\%) = \frac{V_g}{m} \times 100$), the environmental variation coefficient (obtained as $CV_e(\%) = \frac{V_e}{m} \times 100$), and the relative variation coefficient (CVR), represented by the ratio of the genetic variation coefficient to the environmental variation coefficient (CV_g/CV_e). The statistical analysis was performed using RStudio software (R CORE TEAM, 2021).

RESULTS AND DISCUSSION

Based on the results of the variance analyzes from the two experiments, a significant difference ($P \leq 0.05$) was observed among the tomato genotypes for the reproduction factor (FR) as shown in table 2. The coefficients of environmental variation for FR in the first and second experiments were 22% and 17.2%, respectively indicating medium to high precision in environmental control.

The estimates of CVg for the first and second trials were 54.99 and 60.2% for the FR, respectively. These estimates are higher than the environmental coefficient of variation, indicating that genetic variation expressed itself more significantly compared to environmental effects.

The broad-sense heritability for FR in the first and second trials was 91.1% and 94.9%, respectively. The high values of broad-sense heritability emphasized that genetic effects

account for the majority of phenotypic variability. Furthermore, these high heritability values provide greater effectiveness in selecting tomato accessions resistant to *M. incognita* and are a valuable resource for plant breeding programs.

For FR, it was found in both trials that the accessions Ifes 5, Ifes 14, Ifes 23, Ifes 4, Ifes 26, and Ifes 27, along with the commercial cultivars used as controls, 'Grande HT' and 'BRS Portinari,' exhibited $FR < 1.00$, thus being classified as resistant to *M. incognita*, according to the classification of OOSTENBRINK (1966) (Table 3). Notably, the accessions Ifes14 and Ifes26 achieved lower reproduction factors in both trials than the controls 'Grande HT' and 'BRS Portinari,' highlighting the resistance to *M. incognita* observed in these accessions.

PADILLA-HURTADO et al. (2022) reported that genetic resistance is considered the most efficient and sustainable strategy for controlling *Meloidogyne* spp. in tomato, reducing the need for pesticide use. The control cultivar 'Santa Clara' showed a reproduction factor of 72.4 and 68.6% in the first and second experiments; respectively, confirming the viability of the *M. incognita* inoculum used. Furthermore, the classification of accessions regarding resistance is reinforced by the Scott-Knott test, as the accessions considered resistant and the controls belong to statistically distinct groups from the susceptible accessions and the cultivar Santa Clara.

A good correspondence was observed between the classifications obtained in the first and second trials, indicating a consistency in the responses across both trials, which is very valuable information for the improvement of

Table 2 - Analysis of variance showing the mean squares for the reproduction factor (FR) and nematode counts (NC) in the roots of 43 accessions of tomato plants belonging to the Germplasm Bank of Ifes – Campus de Alegre, evaluated in two experiments.

Source of Variation	-----Experiment 1-----			-----Experiment 2-----			-----Jointly-----	
	DF	FR	NC	DF	FR	NC	FR	NC
Genotype	42	8.89 **	7.95 **	42	2.16**	3.60**	-	-
Block	4	1.70	2.70	2	0.08	0.64	-	-
Error	168	0.74	0.97	84	0.17	0.42	-	-
Mean ⁺		28.67	3785.71		5.53	1797.53	17.10	2791.62
CV (%)		22%	13.1%		17.2%	9.3%	19.6%	9.7%
CVg (%)		55.0%	37.5%		60.2%	27.2%	57.6%	32.3%
h ²		91.1%	89.2%		94.9%	89.5%	93.0%	89.3%

CV: coefficient of experimental variation; CVg: coefficient of genetic variation; h²: coefficient of genotypic determination; ** indicates significant effects according to the F test at a significance level of 1%. The analysis used transformed data: $\ln(x+1)$. ⁺ Real mean values (not transformed values).

Table 3 - Means^a of Reproduction factors (FR) of *Meloidogyne incognita* in 43 tomato accessions in two experiments, along with the classification of these accessions regarding their resistance or susceptibility to nematodes according to the classification of OOSTENBRINK (1966).

Accession	-----EXPERIEMENT 1-----		-----EXPERIEMENT 2-----		Jointly
	FR (%)	Classification	FR (%)	Classification	
Ifes1	30,4 a	Susceptible	38,9 a	Susceptible	34,65
Ifes2	48,0 a	Susceptible	42,1 a	Susceptible	45,05
Ifes3	25,7 a	Susceptible	28,9 a	Susceptible	27,30
Ifes4	72,3 a	Susceptible	80,4 a	Susceptible	76,35
Ifes5	0,65 c	Resistant	0,32 c	Resistant	0,49
Ifes6	24,0 a	Susceptible	20,5 a	Susceptible	22,25
Ifes7	44,5 a	Susceptible	41,1 a	Susceptible	42,80
Ifes8	33,8 a	Susceptible	36,4 a	Susceptible	35,10
Ifes9	35,3 a	Susceptible	32,7 a	Susceptible	34,00
Ifes10	2,8 b	Susceptible	4,5 b	Susceptible	3,65
Ifes11	22,7 a	Susceptible	24,6 a	Susceptible	23,65
Ifes12	62,7 a	Susceptible	68,2 a	Susceptible	65,45
Ifes13	51,0 a	Susceptible	54,9 a	Susceptible	52,95
Ifes14	0,32 c	Resistant	0,24 a	Resistant	0,28
Ifes15	55,6 a	Susceptible	50,3 a	Susceptible	52,95
Ifes16	27,3 a	Susceptible	28,0 a	Susceptible	27,65
Ifes17	41,3 a	Susceptible	51,7 a	Susceptible	46,50
Ifes18	12,3 a	Susceptible	8,8 b	Susceptible	10,55
Ifes20	23,7 a	Susceptible	22,3 a	Susceptible	23,00
Ifes21	65,6 a	Susceptible	69,2 a	Susceptible	67,40
Ifes22	41,2 a	Susceptible	43,2 a	Susceptible	42,20
Ifes23	0,32 c	Resistant	0,48 a	Resistant	0,40
Ifes24	0,91 c	Resistant	0,75 c	Resistant	0,83
Ifes26	0,47 c	Resistant	0,36 c	Resistant	0,42
Ifes27	0,42 c	Resistant	0,79 c	Resistant	0,61
Ifes28	3,6 b	Susceptible	4,9 b	Susceptible	4,25
Ifes29	57,1 a	Susceptible	43,7 a	Susceptible	50,40
Ifes30	48,1 a	Susceptible	22,1 a	Susceptible	35,10
Ifes31	42,4 a	Susceptible	50,5 a	Susceptible	46,45
Ifes32	55,0 a	Susceptible	59,4 a	Susceptible	57,20
Ifes33	15,5 a	Susceptible	23,9 a	Susceptible	19,70
Ifes34	26,5 a	Susceptible	20,2 a	Susceptible	23,35
Ifes35	15,0 a	Susceptible	12,8 a	Susceptible	13,90
Ifes36	46,1 a	Susceptible	53,6 a	Susceptible	49,85
Ifes37	33,7 a	Susceptible	39,9 a	Susceptible	36,80
Ifes38	86,8 a	Susceptible	92,4 a	Susceptible	89,60
Ifes39	34,7 a	Susceptible	32,6 a	Susceptible	33,65
Ifes40	58,1 a	Susceptible	63,1 a	Susceptible	60,60
Ifes41	32,7 a	Susceptible	41,7 a	Susceptible	37,20
Ifes42	50,8 a	Susceptible	47,9 a	Susceptible	49,35
Ifes43 (cv. 'Santa Clara')	72,4 a	Susceptible	68,6 a	Susceptible	70,50
Ifes44 (cv. 'Grande HT')	0,53 c	Resistant	0,38 c	Resistant	0,46
Ifes45 (cv. 'BRS Portinari')	0,71 c	Resistant	0,62 c	Resistant	0,67

^aMeans followed by the same letter in the column do not differ by the Scott-Knott test at 5% probability. Means are expressed based on untransformed data.

tomato cultivation. The reproduction values for most accessions were similar in both trials. CASTAGNONE-SERENO (2006), KALKMANN et al. (2013) and PADILLA-HURTADO et al. (2022) reported that variations in reproductive rates may be partially due to genetic factors that confer resistance or susceptibility to the host, variations in nematode populations, or elevated temperatures.

It is important to note that none of the evaluated tomato accessions completely prevented nematode reproduction (FR=0), indicating that they are not immune to *M. incognita*. This shows how sensitive the tomato crop can be to the presence of this phytoparasitic nematode. SILVA et al. (2019) describe that since this type of resistance is not complete, it further increases the need to employ other control strategies alongside the use of resistant genotypes.

SILVA et al. (2019), while working on tomato improvement for resistance to root-knot nematodes, also obtained results similar to those of the present study. The authors evaluated 102 commercial and wild tomato genotypes in two trials and found three accessions resistant to *M. enterolobii*, one resistant to *M. incognita*, and one resistant to *M. javanica*. They emphasized the importance of finding sources of resistance to nematodes in tomato cultivation for plant improvement, the continuous breakdown of resistance in the crop, and the efforts in introgressing these genes through crossings. Other authors studying tomatoes and other vegetables have reported not observing resistant genotypes to *Meloidogyne* in their studies, highlighting the difficulty in obtaining resistant genotypes to the pathogen and its great capacity to overcome plant resistance (FRANZENER et al., 2007; MOLINARI et al., 2010; PINHEIRO et al., 2014; FERNANDES et al., 2014).

For the reproduction index (IR), all five classes established by TAYLOR's classification (1967) were identified (Table 4). The observed results align with OOSTENBRINK'S classification (1966). The accessions Ifes5, Ifes14, Ifes23, Ifes24, Ifes26, and Ifes27, along with the commercial cultivars 'Grande HT' and 'BRS Portinari,' which were classified as resistant according to Oostenbrink, were categorized as either very resistant (MR) or highly resistant/immune (AR/I). MARCHESE et al. (2010) suggested that in the selection process, attention should be given only to accessions that exhibit AR/I and MR reactions, making them suitable for selection.

SUJATHA et al. (2017), while evaluating 40 tomato accessions in India using the reproduction index as a criterion, also observed the occurrence of

all five classes, identifying five genotypes resistant to *M. incognita*. Similarly, EL-SAPPAH et al. (2022), in their evaluation of 13 tomato genotypes in China, found three accessions resistant to *M. incognita*, also utilizing the reproduction index.

The control cultivar 'Santa Clara' was classified as susceptible (100% for the reproduction index), while accession Ifes 38 exhibited an IR greater than that of the control, indicating it is even more susceptible. For most of the evaluated accessions, a consistency in the responses for the reproduction index was observed in both the first and second trials, with no significant discrepancies between the classifications. As with Oostenbrink's classification, the Scott-Knott test provided the formation of statistically distinct groups for the resistant and susceptible accessions.

Both classifications used were considered effective in discriminating accessions as resistant or susceptible, showing good similarity between the results of the two trials for both classification types. However, OOSTENBRINK'S classification (1966), which utilizes the reproduction factor, is easier to apply as it categorizes accessions into just two levels (resistant or susceptible). Additionally, the data can be used in a more straightforward and immediate manner, relying solely on the ratio of final to initial nematode egg counts obtained from the tomato accessions.

TAYLOR'S classification (1967), which employs the reproduction index, also proved viable and effective for evaluating tomato materials. However, due to the greater number of classes, a more detailed analysis of the results is required. In both classifications, no differences were observed between the classifications of the resistant controls and the susceptible control, reinforcing the thoroughness of the methodology used. These classifications have been employed by various authors working on nematode resistance in tomatoes (JAITEH et al., 2012; GHARABADIYAN et al., 2013; SUJATHA et al., 2017; EL-SAPPAH et al., 2022). Additionally, the integration of molecular techniques—which have shown increasingly promising potential (ZAHOR et al., 2025)—with phenotypic evaluation methods, such as those employed in this study, is expected to support the identification of nematode-resistant tomato genotypes in breeding programs.

CONCLUSION

According to the two classifications discussed in this study and across both experiments,

Table 4 - Means* of Reproduction indices (IR%) of *Meloidogyne incognita* in 43 tomato accessions i in two experiments, along with the classification of these accessions regarding their resistance or susceptibility to nematodes according to the classification of TAYLOR (1967).

Accession	-----EXPERIEMENT 1-----		-----EXPERIEMENT 2-----	
	IR (%)	Classification	IR (%)	Classification
Ifes1	41,9 a	LR	56,7a	Susceptible
Ifes2	66,3 a	Susceptible	61,3 a	Susceptible
Ifes3	35,5 a	Susceptible	42,1 a	LR
Ifes4	72,3 a	Susceptible	117,2 c	Susceptible
Ifes5	0,90 a	AR/ I	0,47 a	AR/ I
Ifes6	33,1 a	LR	29,9 a	LR
Ifes7	61,5 a	Susceptible	41,1 a	LR
Ifes8	46,7 a	LR	59,9 a	Susceptible
Ifes9	48,7 a	LR	47,7 a	LR
Ifes10	3,9 a	MR	6,5 a	MR
Ifes11	31,3 a	LR	35,9 a	LR
Ifes12	86,6 b	Susceptible	99,4 c	Susceptible
Ifes13	70,4 b	Susceptible	80,0 b	Susceptible
Ifes14	0,44 a	AR/ I	0,35 a	AR/ I
Ifes15	76,8 b	Susceptible	72,9 b	Susceptible
Ifes16	37,7 a	LR	40,8 a	LR
Ifes17	57,0 a	Susceptible	75,3 a	Susceptible
Ifes18	16,9 a	MoR	12,8 a	MoR
Ifes20	32,7 a	LR	32,5 a	LR
Ifes21	90,6 c	Susceptible	100,8 c	Susceptible
Ifes22	56,9 a	Susceptible	63,0 a	Susceptible
Ifes23	0,44 a	AR/ I	0,69 a	AR/ I
Ifes24	1,2 a	MR	1,09 a	MR
Ifes26	0,47 a	AR/ I	0,52 a	AR/ I
Ifes27	0,65 a	AR/ I	1,15 a	MR
Ifes28	5,0 a	MR	7,1 a	MR
Ifes29	78,9 b	Susceptible	63,7 a	Susceptible
Ifes30	66,4 a	Susceptible	32,2 a	Susceptible
Ifes31	58,5 a	Susceptible	73,6 b	Susceptible
Ifes32	75,9 b	Susceptible	86,6 b	Susceptible
Ifes33	21,4 a	MoR	34,8 a	LR
Ifes34	36,6 a	LR	29,4 a	LR
Ifes35	20,7 a	MoR	18,0 a	MoR
Ifes36	63,7 a	Susceptible	78,1 b	Susceptible
Ifes37	46,5 a	LR	58,2 a	Susceptible
Ifes38	119,9 c	Susceptible	134,7 c	Susceptible
Ifes39	47,9 a	LR	47,5 a	LR
Ifes40	80,2 b	Susceptible	92,0 c	Susceptible
Ifes41	45,1 a	LR	60,8 a	Susceptible
Ifes42	70,2 a	Susceptible	69,8 a	Susceptible
Ifes43 (cv. 'Santa Clara')	100 c	Susceptible	100 c	Susceptible
Ifes44 (cv. 'Grande HT')	0,73 a	AR/ I	0,55 a	AR/ I
Ifes45 (cv. 'BRS Portinari')	0,98 a	AR/ I	0,90 a	AR/ I

*Means followed by the same letter in the column do not differ by the Scott-Knott test at 5% probability; Means are expressed based on untransformed data; IR above 51% = susceptible; LR = slightly resistant, IR from 26 to 50%; MoR = moderately resistant, IR from 11 to 25%; MR = very resistant, IR from 1 to 10%; AR/I = highly resistant/immune, IR below 1%.

the accessions considered resistant to *M. incognita* are: Ifes 5, Ifes 14, Ifes 23, Ifes 24, Ifes 26, and Ifes 27, along with the commercial cultivars ‘Grande HT’ and ‘BRS Portinari’. This information is valuable for tomato improvement programs.

ACKNOWLEDGMENTS

The authors would like to thank the Fundação de Apoio à Pesquisa do Espírito Santo (FAPES) and the Instituto Federal do Espírito Santo (IFES) for their support and scholarship grants for this research and was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Brasil - Finance code 001. TOS was supported by Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ – Grant no. E-26/200.036/2024 and E-26/200.037/2024).

DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

AUTHORS’ CONTRIBUTIONS

All authors contributed equally for the conception and writing of the manuscript. All authors critically revised the manuscript and approved of the final version.

DATA AVAILABILITY STATEMENT

The data of this manuscript is available directly with the author or from a permanent repository.

DECLARATION OF USE OF ARTIFICIAL INTELLIGENCE

Artificial intelligence tools were not used to write the manuscript or to replace the authors’ activities and skills, such as preparing the abstract, keywords, hypotheses, and conclusion of the study, among other aspects of the manuscript.

REFERENCES

ARAUJO, E. F. et al. Physiological and sanitary quality of organic tomato seeds treated with clove basil extracts. **Comunicata Scientiae**, v.9, n.1, p.26-33, 2018. Available from: <<https://doi.org/10.14295/cs.v9i1.913>>. Accessed: Jul. 10, 2022. doi: 10.14295/cs.v9i1.913.

ASHRAFI, H. et al. A new genetic linkage map of tomato based on a *Solanum lycopersicum* × *S. pimpinellifolium* RIL population displaying locations of candidate pathogen response genes. **Genome**, v.52, n.11, p.935-956, 2009. Available from: <<https://doi.org/10.1139/G09-065>>. Accessed: Apr. 22, 2022. doi: 10.1139/g09-065.

ÁVILA JÚNIOR, J. H. de. **Reações de genótipos de tomateiro industrial a *Meloidogyne incognita* e eficiência de métodos**

de controle. 2017. 62 f: il. Dissertação (Mestrado Profissional em Olericultura) - Instituto Federal Goiano, Morrinhos, 2017. Available from: <https://repositorio.ifgoiano.edu.br/bitstream/prefix/94/1/DISSERTA%C3%87%C3%83O_OLERICULTURA_JOSE%20AVILA%20JUNIOR.pdf>. Accessed: Jul. 10, 2022.

BALBINO, J. M. et al. Produção integrada de tomate de mesa no Espírito Santo. 2014. In: **Relatório do Ministério da Agricultura, Pecuária e Abastecimento**. Produção integrada no Brasil: agropecuária sustentável, alimentos seguros, Brasília, DF, 2009. p.851-866. Available from: <<http://biblioteca.incaper.es.gov.br/digital/handle/item/336>>. Accessed: Apr. 22, 2022.

BONETTI, J. I. S.; FERRAZ, S. Modificação do método de Hussey e Barker para extração de ovos de *Meloidogyne exigua* de raízes de cafeeiro. **Fitopatologia Brasileira**, v.6, n.3, p.553, 1981. Available from: <<https://www.scienceopen.com/document?vid=9232a12b-2a57-4b2b-8724-dae6ad63e065>>. Accessed: Jul. 08, 2022.

CAMPOS, M. D. et al. High throughput sequencing unravels tomato-pathogen interactions towards a sustainable plant breeding. **Horticulture research**, v.8, 2021. Available from: <<https://doi.org/10.1038/s41438-021-00607-x>>. Accessed: Jan. 08, 2023. doi: 10.1038/s41438-021-00607-x.

CASTAGNONE-SERENO, P. Variabilidade genética e evolução adaptativa em nematoides partenogenéticos das galhas. **Hereditariedade**, v.96, p.282-28, 2006. Available from: <<https://doi.org/10.1038/sj.hdy.6800794>>. Accessed: Jan. 06, 2023. doi: 10.1038/sj.hdy.6800794.

CHARCHAR, J. M.; LOPES, C. A. Nematoides. In: LOPES, C. A.; ÁVILA, A. C. de. **Doenças do tomateiro**. Brasília: Embrapa Hortaliças, 2005. Available from: <<https://www.infoteca.cnptia.embrapa.br/handle/doc/778171>>. Accessed: Jan. 08, 2023.

COMPANHIA NACIONAL DE ABASTECIMENTO - CONAB. Available from: <<https://www.conab.gov.br/info-agro/safra/progresso-de-safra>>. Accessed: Aug. 27, 2024.

DE PAULA, E. et al. Genetic Diversity of Common Bean (*Phaseolus vulgaris* L.) Landraces Based on Morphological Traits and Molecular Markers. **Plants**, v.13, n.18, p.2584, 2024. Available from: <<https://doi.org/10.3390/plants13182584>>. Accessed: Sept. 30, 2024. doi: 10.3390/plants13182584.

EL-SAPPAH, A. H. et al. Identification of novel root-knot nematode (*Meloidogyne incognita*) resistant tomato genotypes. **JAPS: Journal of Animal & Plant Sciences**, v.32, n.1, 2022. Available from: <<https://doi.org/10.36899/JAPS.2022.1.0407>>. Accessed: Nov. 17, 2023. doi: 10.36899/JAPS.2022.1.0407.

ESBENSHADE, P. R.; TRIANTAPHYLLOU, A. C. Isozyme phenotypes for the identification of *Meloidogyne* species. **Journal of Nematology**, v.22, n.1, p.10-15, 1990. Available from: <<https://pmc.ncbi.nlm.nih.gov/articles/PMC2619005/>>. Accessed: Feb. 22, 2021.

FERNANDES, R. H. et al. “*Pochonia chlamydosporia* e *Bacillus subtilis* no controle de *Meloidogyne incognita* e *M. javanica* em mudas de tomateiro.” **Bioscience Journal**, v.30, n.1, p.34-38, 2014. Available from: <<https://pesquisa.bvsalud.org/portal/resource/pt/biblio-946990>>. Accessed: Jul. 08, 2022.

FERNANDES, M. D. O. et al. Morpho-agronomic characterization and analysis of genetic divergence among accessions of tomatoes (*Solanum lycopersicum* L.). **Ciência Rural**, v.48, p.11, 2018.

- Available from: <<https://doi.org/10.1590/0103-8478cr20180433>>. Accessed: Jan. 08, 2023. doi: 10.1590/0103-8478cr20180433.
- FRANZENER, G. et al. Proteção de tomateiro a *Meloidogyne incognita* pelo extrato aquoso de *Tagetes patula*. **Nematologia Brasileira**, v.31, p.27-36, 2007. Available from: <<https://www.cabidigitallibrary.org/doi/pdf/10.5555/20073136750>>. Accessed: Jul. 10, 2022. doi: 10.4322/nematologia.v31.n1.10210.
- GARBOWICZ, K. et al. Quantitative trait loci analysis identifies a prominent gene involved in the production of fatty acid-derived flavor volatiles in tomato. **Molecular Plant**, v.11, n.9, p.1147-1165, 2018. Available from: <<https://pubmed.ncbi.nlm.nih.gov/29960108/>>. Accessed: Jul. 08, 2022. doi: 10.1016/j.molp.2018.06.003.
- GHARABADIYAN, F. Determining of root-knot nematode (*Meloidogyne javanica*) damage function for tomato cultivars. **Journal of Agricultural Sciences**, v.58, n.2, p.147-157, 2013. Available from: <10.2298/JAS1302147G>. Accessed: Jan. 06, 2023. doi: 10.2298/JAS1302147G.
- HUSSEY, R. S.; BARKER K. R. A comparison of methods of collecting inocula of *Meloidogyne* spp. including a new technique. **Plant Disease Reporter**, v.57, p.1025-1028, 1973. Available from: <<https://www.cabidigitallibrary.org/doi/full/10.5555/19740810950>>. Accessed: Feb. 22, 2021.
- JAITEH, F. et al. Evaluation of tomato genotypes for resistance to root-knot nematodes. **African Crop Science Journal**, v.20, n.1, 2012. Available from: <<https://www.ajol.info/index.php/acsj/article/view/78717>>. Accessed: Jul. 10, 2022. doi: 10.4314/ajcs.v20i1.2.
- JATALA, P.; BRIDGE, J. Nematode parasites of root and tuber crops. **Plant parasitic nematodes in subtropical and tropical agriculture**, v.137, n.180, 1990. Available from: <https://horizon.documentation.ird.fr/exl-doc/pleins_textes/2021-09/34395.pdf>. Accessed: Sept. 30, 2024.
- JENKINS, W. R. A rapid centrifugal-flotation technique for separating nematodes from soil. **Plant Disease Reporter**, v.48, p.692, 1964. Available from: <<https://www.cabidigitallibrary.org/doi/full/10.5555/19650801105>>. Accessed: Sept. 30, 2024.
- KALKMANN, D. C. et al. Reaction of sweet potato clones to *Meloidogyne incognita* races 1 and 4, and estimation of genetic parameters. **Horticultura Brasileira**, v.31, p.293-296, 2013. Available from: <<https://doi.org/10.1590/S0102-05362013000200019>> Accessed: Jul. 08, 2022. doi: 10.1590/S0102-05362013000200019.
- MARCHESE, A. et al. Seleção de clones de batata-doce resistentes a *Meloidogyne incognita* raça 1. **Pesquisa Agropecuária Brasileira**, v.45, p.997-1004, 2010. Available from: <<https://doi.org/10.1590/S0100-204X2010000900009>>. Accessed: Jul. 10, 2022. doi: 0.1590/S0100-204X2010000900009.
- MOLINARI, S.; BASER, N. Induction of resistance to root-knot nematodes by SCAR elicitors in tomato. **Crop Protection**, v.29, p.1354-1362, 2010. Available from: <<https://doi.org/10.1016/j.cropro.2010.07.012>>. Accessed: Apr. 22, 2022. doi: 10.1016/j.cropro.2010.07.012.
- OOSTENBRINK, M. **Major characteristics of the relation between nematodes and plants**. Mededelingen van de landbouwhogeschool te Wageningen. v.66, p.1-46, 1966. Available from: <<https://research.wur.nl/en/publications/major-characteristics-of-the-relation-between-nematodes-and-plant/>>. Accessed: Apr. 22, 2022.
- PADILLA-HURTADO, B. et al. Evaluation of root-knot nematodes (*Meloidogyne* spp.) population density for disease resistance screening of tomato germplasm carrying the gene Mi-1. **Chilean journal of agricultural research**, v.82, n.1, p.157-166, 2022. Available from: <<http://dx.doi.org/10.4067/S0718-58392022000100157>>. Accessed: Jan. 06, 2023. doi: 10.4067/S0718-58392022000100157.
- PINHEIRO, J. B. et al. **Identificação de espécies de *Meloidogyne* em tomateiro no Brasil**. Brasília: Embrapa Hortaliças, 2014a (Boletim de Pesquisa e Desenvolvimento n. 102). Available from: <<https://www.infoteca.cnptia.embrapa.br/bitstream/doc/992143/1/1244BPD102.pdf>>. Accessed: Apr. 22, 2022.
- PHILBRICK, A. N. et al. *Meloidogyne enterolobii*, a major threat to tomato production: current status and future prospects for its management. **Frontiers in plant science**, v.11, 606395, 2020. Available from: <<https://doi.org/10.3389/fpls.2020.606395>>. Accessed: Jan. 06, 2023. doi: 10.3389/fpls.2020.606395.
- CORE TEAM. **R: A Language and Environment for Statistical Computing**; R Foundation for Statistical Computing: Vienna, Austria, 2021; Available from: <<https://www.R-project.org/>>. Accessed: Jan. 08, 2023.
- SILVA, A. J. D. et al. Search for sources of resistance to *Meloidogyne enterolobii* in commercial and wild tomatoes. **Horticultura Brasileira**, v.37, n.2, p.188-198, 2019. Available from: <<https://doi.org/10.1590/S0102-053620190209>>. Accessed: Apr. 22, 2022. doi: 10.1590/S0102-053620190209.
- SUJATHA, R. et al. Screening of tomato genotypes for root knot nematode (*Meloidogyne incognita* Kofoid and White Chitwood). **International Journal of Current Microbiology and Applied Sciences**, v.6, n.3, p.1525-1533, 2017. Available from: <<https://doi.org/10.1590/S0102-053620190209>>. Accessed: Apr. 22, 2022. doi: 10.20546/ijemas.2017.603.175.
- TAYLOR, A. L. **Introduction to research on plant nematology**. In: **FAO guide to study and control of the plant-parasitic nematodes**. Rome: Food And Agricultural Organization of the United Nations. 133p, 1967.
- TIEMAN, D. et al. A chemical genetic roadmap to improved tomato flavor. **Science**, v.355, n.6323, p.391-394, 2017. Available from: <<https://doi.org/10.1126/science.aal1556>>. Accessed: Jul. 10, 2022. doi: 10.1126/science.aal1556.
- TREICHEL, B. et al. **Rhizobia Increase the Yield and Vegetal Growth of Different Tomato Varieties Under Greenhouse and Field Conditions**, 2016. Available from: <https://papers.ssrn.com/sol3/papers.cfm?abstract_id=4220807>. Accessed: Apr. 22, 2022. doi: 10.2139/ssrn.4220807.
- WANG, Y. et al. The genomic route to tomato breeding: past, present, and future. **Plant Physiology**, v.195, n.4, 2024. Available from: <<https://doi.org/10.1093/plphys/kiae248>>. Accessed: Sept. 30, 2024. doi: 10.1093/plphys/kiae248.
- ZAHOOR, A. et al. Assessment of tomato germplasms for Mi-1.2-mediated resistance to *Meloidogyne incognita*: Integrating in-silico dynamics and bioinformatic approaches. **Physiological and Molecular Plant Pathology**, p.102714, 2025. Available from: <<https://doi.org/10.1016/j.pmp.2025.102714>>. Accessed: Jul. 14, 2025. doi: 10.1016/j.pmp.2025.102714.