RESEARCH

Evaluation of root-knot nematodes (*Meloidogyne* spp.) population density for disease resistance screening of tomato germplasm carrying the gene *Mi-1*

Beatriz Padilla-Hurtado[1, 3], Yacenia Morillo-Coronado[2], Sandra Tarapues[1], Santiago Burbano[1], Mauricio Soto-Suárez[2], Ramiro Urrea[1], and Nelson Ceballos-Aguirre[1*]

[1]Universidad de Caldas, Doctorado en Ciencias Agrarias, Facultad de Ciencias Agropecuarias, Calle 65 No. 26-10, Manizales, Colombia.
[*]Corresponding author (nelson.ceballos@ucaldas.edu.co).
[2]Corporación Colombiana de Investigación Agropecuaria-AGROSAVIA, km 14 Vía Mosquera, Bogotá, Colombia.
[3]Universidad Católica de Manizales, Instituto de Investigaciones en Microbiología y Biotecnología Agroindustrial, Carrera 23 N° 60-63. Manizales, Colombia.

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ABSTRACT

The root-knot nematode *Meloidogyne* spp. causes yield losses of up to 68% on tomato (*Solanum lycopersicum* L.) crops. Genetic resistance in the host plant makes the crop sustainable and it can breakdown when there is a high population density of the pathogen. The objective of this study was to determine the nematode population density that allow determining the resistance potential of tomato germplasm associated with the *Mi-1* resistance gen. The *Mi-1* gene was evaluated with the molecular marker SCAR Mi-23 and specific primers in the genotypes COLY007, IAC1687, LA0445, IAC1622 and two commercial controls (susceptible and resistant). The damage scale and the number of individuals recovered (eggs and juveniles) were assessed, with different population densities of the pathogen inoculated (0, 1000, 2000, 3000, 4000 and 5000 individuals plant^{-1}), in a split plot design, with six replicates and a plant as the experimental unit. The genotype IAC1687 and the resistant commercial control presented the resistance allele of the *Mi-1* gene and were classified as moderately resistant to a density of 1000 individuals plant^{-1}. Despite having the *Mi-1* gene, the COLY007 genotype was classified as moderately susceptible and with densities greater than 1000 individuals plant^{-1} can break resistance in all genotypes evaluated. Additionally, it is necessary to correlate the genotypic and phenotypic responses to guarantee the success of the selection supported by molecular markers such as SCAR Mi-23 and identify promising genotypes that could be included in a long-term breeding and also used as rootstocks in an integrated management of root-knot nematode.

Key words: Genetic resistance, genotype-phenotype association, Mi-23 molecular marker, radical nodulation susceptibility.

INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is a plant native to South and Central America. Worldwide, there are 4.7 million hectares of production, in which 243 million tons produced were reported for 2018 (FAO, 2020). Among the soil-borne diseases affecting tomato crop, one of the most important is root-knot nematode (RKN) caused by *Meloidogyne* spp. Root nodulation causes significant economic losses, reducing yield by up to 68% (Salazar-Antón and Guzmán-Hernández, 2013; Khan et al., 2019). *Meloidogyne incognita*, *M. arenaria*, *M. hapla* and *M. javanica*, which are part of the *Meloidogyne* spp. complex, are among the top 10 most devastating phytoparasitic nematodes of economically important crops (Jones et al., 2013).

After penetrate the roots, *Meloidogyne* spp. moves to the vascular bundles to find a successful feeding site (Rodiuc et al., 2014). This process leads to the formation of nodules or galls, which affect the uptake of nutrients and water by the plant (Dagatti et al., 2014). As a consequence of root gall formation, plants can manifest secondary symptoms such as wilting, chlorosis in the oldest leaves, general reduction of plant growth, floral abortions, decrease in both fruit quality and fresh weight, as well as a reduction in the number of fruits. Following senescence and death of the plant in severe infections (Ortiz et al., 2015).

The polyphagous nature of the root-knot nematode *Meloidogyne* spp. poses severe constraints for an effective management strategy of the disease management practices such as the use of chemical nematicides, integration of cover crops into the cropping system, the use of rootstocks with resistance to root nodulation and biological control have been documented (Adam et al., 2014). However, factors such as the toxic effect of chemical nematicides on the environment and the wide range of hosts of *Meloidogyne* spp. as well as the effect of soil properties limit the use of these practices (Barbary et al., 2015). The use of genetic resistance is a highly efficient way of controlling the disease with a positive impact in both ecological and economic terms (Cervantes-Moreno et al., 2014; Cardoso et al., 2019).

Bailey in 1941 was the first to report genetic resistance in tomato to the root-knot nematode (RKN), in the wild relative *Solanum peruvianum* (Bailey, 1941). This resistance was later attributed to a single dominant gene named as *Mi-1*, located on chromosome 6 of this species *S. peruvianum* (Pérez-Almeida et al., 2016). The *Mi-1* gene encodes an R protein of the type nucleotide-binding site and leucine-rich repeat domains (NBS-LRR) and confers resistance to three of the four species of *Meloidogyne* spp. that are economically devastating for the tomato *M. incognita*, *M. arenaria*, and *M. javanica* but not for *M. hapla* (Aydinli and Mennan, 2019). Two homologues of this gene, *Mi-1.1* and *Mi-1.2*, were identified in the *Mi* locus. Only *Mi-1.2* confers resistance to the RKN in tomato plants; other resistance genes (*Mi-2*, *Mi-3*, *Mi-4*, *Mi-5*, *Mi-6*, *Mi-7*, *Mi-8*, *Mi-HT*, and *Mi-9*) have also been identified in different species of wild tomato. Wild tomato has the ability to generate variation in the locus of resistance to nematodes, which leads to the generation of new sources of resistance (El-Sappah et al., 2019).

Resistant tomato varieties with the gene *Mi-1* show a decrease by 85% in the reproduction of *Meloidogyne* spp. compared to susceptible varieties. However, the effectiveness of this resistance can be associated with different factors, such as, pathogen population density, the state of homozygous and heterozygous of the resistance loci and soil temperature; temperatures greater than 28 °C result in a decrease of the effectiveness of *Mi-1* gene (Özalp and Devran, 2018; Chidichima et al., 2021). High initial population densities not only affect crop yield and fruit quality, but interfere with the level of resistance conferred by the *Mi-1* gene. Results presented by Maleita et al. (2012) suggest that the *Mi-1* gene only provides partial resistance; in the scenario of high population densities, the plant and root growth is reduced and reproductive parameters for the nematodes increase.

Most of the diversity of tomato is found in its wild forms, the cultivated tomato has a low diversity and it is estimated that it contains less than 5% of the genetic variation of its wild relatives (Mata-Nicolás et al., 2020). The cherry tomato is considered the first domesticated form of the tomato, which was derived from *S. pimpinellifolium*, species native to the coastal regions of Ecuador and Peru, and has been reported growing in sympatry with the wild relatives *S. peruvianum* and *S. hirsutum*, and with *S. l. cerasiforme* and *S. lycopersicum*, which implies a genetic exchange between species that share a geographic region, which was confirmed by the presence of a genotypic mixture between cultivated and wild tomatoes (Ranc et al., 2012). It is necessary to incorporate promising wild species in breeding programs for the cultivated species, since they are sources of variability and possess potential genes to get into cultivated species. Knowledge of genetic diversity is of vital importance for the search for solutions to problems that include plant breeding activities in order to seek the development of varieties of high quality, yield, resistant to biotic factors and abiotic factors, among other characteristics of economic importance (Herison et al., 2018).

With the evidence of introgression in tomato species that share the same natural geographic distribution and origin, it is possible that the acquisition of nematode resistance genes might have effect on the fitness and therefore, the search for the resistance gene *Mi-1* can be done with molecular markers in plants of different species of the genus *Solanum*. The Mi-23 molecular marker linked to the gene *Mi-1.2* is the codominant marker type sequenced characterized amplified region (SCAR), it amplifies a fragment of approximately 450 bp in susceptible plants of *S. lycopersicum* (*mi*/*mi*), a fragment of 400 bp in resistant phenotypes (*Mi*/*Mi*), and both fragments in heterozygous plants (*Mi*/*mi*) (Pérez-Almeida et al., 2016).

The objective of this study was to determine the population density of nematodes that allows evaluating the resistance potential of tomato germplasm associated with the codominant SCAR marker Mi-23 to identify moderately resistant and highly resistant genotypes that can be included in genetic improvement programs for the RKN management.

MATERIAL AND METHODS

Meloidogyne spp. inoculum

The inoculum of *Meloidogyne* spp. was obtained from roots with nodulations of tomato plants affected by the pathogen from La Parroquia farm (5°2'2.4'' N, 73°40'37.2'' W), Palestina, Caldas, Colombia. The extraction of eggs and juveniles from the nematode was performed using the sugar flotation technique described by Jenkins (1964), from which the population densities to be evaluated were adjusted.

Plant material

Six genotypes of tomato (*Solanum lycopersicum* L.), four wild of *Solanum* spp. of the Germplasm Bank from Universidad Nacional of Colombia (Palmira) and two commercial controls, one susceptible and one resistant (with *Mi-1* gene), were evaluated (Table 1). The phenotyping was carried out in the Montelindo farm of the University of Caldas, located in Palestina (5°05' N, 75°40' W; 1010 m a.s.l.), Caldas. This location has an average temperature of 22.8 °C, annual average rainfall of 2200 mm, annual solar brightness of 2010 h and 76% of average relative humidity.

Germination was carried out in trays of 128 locules with Sphagnum grade 3 peat substrate. After 30 d, the transplant was carried out in 8 kg polyethylene bags with soil previously disinfected with dazomet (500 g m^{-3}; 3,5-dimethyl-1,3,5-thiadiazinane-2-thione, BASF, Bogotá, Colombia). These were located in a semi-controlled system with beds raised 1 m from the ground and were inoculated with different population densities of the nematode (0, 1000, 2000, 3000, 4000 and 5000 individuals $plant^{-1}$, respectively) at 15 d after transplanting.

Amplification of the molecular marker SCAR Mi-23

For the amplification of the molecular marker type SCAR Mi-23 associated with the *Meloidogyne* spp. resistance gene (*Mi-1*), 50 ng genomic DNA were extracted from young leaf tissue of each tomato genotype, respectively following the gDNA extraction protocol by Doyle and Doyle (1990). The primers Mi-23F: 5'TGGAAAAATGTTGAATTTCTTTTG3' and Mi-23R: 5'GCATACTATATGGCTTGTTTACCC3' were used following the amplification conditions reported by Pérez-Almeida et al. (2016). Briefly, 25 µL PCR mixture containing 3 mM $MgCl_2$, 10X Buffer, 400 µM DNTPs, 10 µM each primer, and 1 U Taq polymerase (Bioline, London, UK) were prepared. The amplification program used was as follows: DNA denaturation at 94 °C for 5 min, followed by 35 cycles of amplification (denaturation at 94 °C for 30 s, alignment of the primers at 54 °C for 30 s and extension at 72 °C for 1 min) and a final extension step at 72 °C for 7 min. The amplification products were observed in a 1.5% agarose gel stained with GelRed (Biotium, Fremont, California, USA) using the molecular weight marker 1 kb plus (Promega, Madison, Wisconsin, USA) to determine the size of the amplified fragments.

To verify the presence of the *Mi-1* gene in tested genotypes with the Mi-23 molecular marker, *Mi-1* gene-specific primers reported by De Carvalho et al. (2015) (C2S4 5'-CTAAGAGGAATCTCATCACAGG-3'/VIGS_F 5'-CTTGCGTCTACTGACTCTTTCC-3') were used to amplify DNA fragments of 359 pb with amb Taq DNA polymerase (Bioline), following manufacturer's instructions. Thermal cycling conditions were 94 °C for 2 min, followed by 34 cycles of 94 °C for 30 s, 52 °C for 30 s, and 72 °C for 5 min.

Table 1. Genotypes evaluated in this study.

Code	Origin	Species
IAC1622	Brazil	*Solanum lycopersicum* L. var. *cerasiforme* (Alef.) Voss
COLY007	Colombia	*S. lycopersicum* var. *cerasiforme*
LA0445	Peru	*S. peruvianum* L.
IAC1687	Brazil	*S. lycopersicum* var. *cerasiforme*
Susceptible genotype	Commercial	*S. lycopersicum* L.
Resistant genotype	Commercial	*S. lycopersicum*

IAC: Accessions from the Agronomic Institute of Campinas, Campinas, Brazil; COLY: accessions from Colombia (Herbarium of the University of Caldas); LA: accessions from the Tomato Genetic Resources Center (TGRC), University of California-Davis.

Experimental design

A split plot design was used, the main plot being the population density of the nematode (0, 1000, 2000, 3000, 4000 and 5000 individuals plant^{-1}) and the genotype the secondary plot, with six replicates per combination and one plant as experimental unit.

Variables

Sixty days post inoculation, damage scale (DS) was evaluated using the severity scale proposed by Taylor and Sasser (1983) (Table 2), and total population of nematodes (TPN) quantifying the number of individuals (eggs and juveniles) in 100 g root using optical microscopy (40X), as well as agronomic variables such as: number of fruits (NF), fruit weight (FW, g) per plant, main root length (RL, cm), stem length (SL, cm) defined as the length from the base of the stem to the apical part of the plant, fresh weight of roots (FWR, g), fresh weight of the aerial part of the plant (FWA, g), dry weight of roots and aerial part of the plant (DWR and DWA, respectively; g).

Statistical analyses

ANOVA and Duncan-type mean tests were performed using the GLM procedure to determine the occurrence of significant differences in genotypes, evaluated doses and their interaction at a significant level $p \leq 0.05$. For the relationship of the plant development variables with the damage incidence variables, Pearson and Kruskal-Wallis comparison tests were performed ($p \leq 0.05$).

RESULTS AND DISCUSSION

SCAR Mi-23 molecular marker amplification

The *Mi-1* resistance allele evaluated through the SCAR Mi-23 molecular marker was amplified in three of the six genotypes evaluated with an amplification product of approximately 400 bp. Genotype IAC1687 was homozygous dominant (*Mi*/*Mi*) and genotypes COLY007 and the commercial resistant genotype, were heterozygous (*Mi*/*mi*) with amplification of the resistance band (400 bp) and the susceptibility band (450 bp) (Figure 1). The susceptibility allele was found in genotypes LA0445, IAC1622 and the susceptible commercial material (*mi*/*mi*), with an amplification product of approximately 450 bp (Figure 1).

The gene *Mi-1* has been successfully incorporated into many commercially available tomato varieties and is the most studied resistance source (Seid et al., 2015). It has been reported that the expression of the gene *Mi-1* is affected by factors such as high soil temperature (> 28 °C) (de Carvalho et al., 2015), virulent populations of *Meloidogyne* spp. to the tomato gene *Mi-1*, and high population densities of the pathogen (Maleita et al., 2012). Despite the presence of the *Mi-1* gene, the expression of genotype resistance was affected by the evaluation conditions, as evidenced below in the phenotypic response.

Phenotypic response to different population densities of *Meloidogyne* spp.

The genotypes evaluated with different population densities of *Meloidogyne* spp. showed root nodulation symptoms in all population densities above 1000 individuals plant^{-1}, in contrast to uninoculated plants. In this population density 1000 individuals plant^{-1}, IAC1687 genotype and resistant commercial genotype presented the highest number of experimental units in grade 1 and 2 damage scale classified as resistant and moderately resistant respectively. The accession LA0445, despite being *S. peruvianum*, did not amplify the molecular marker Mi-23, phenotypically it showed a behavior like

Table 2. Damage scale to evaluate the phenotypic response in tomato plants against *Meloidogyne* spp.

Degree of damage[1]	Percentage of damage[1]	Phenotypic response[2]
G1	0-10	Resistant
G2	11-25	Moderately resistant
G3	26-50	Moderately susceptible
G4	51-75	Susceptible
G5	76-100	Highly susceptible

[1]Degree and percentage of damage by Taylor and Sasser's scale (Taylor and Sasser, 1983).
[2]Genotype response proposed by Sañudo et al. (2003).

the susceptible control. The accession COLY007 despite having amplified the molecular marker Mi-23, phenotypically showed a behavior similar to the susceptible control (Figure 2, Table 2). This requires genotypic and phenotypic correlation studies that guarantee the satisfactory response of the evaluated genotypes, in accordance with the variables and objectives proposed at the beginning of the breeding program.

Figure 1. A. Amplification of the SCAR *Mi*-23 molecular marker in six tomato genotypes. B. *Mi-1* gene-specific primers (C2S4/VIGS_F).

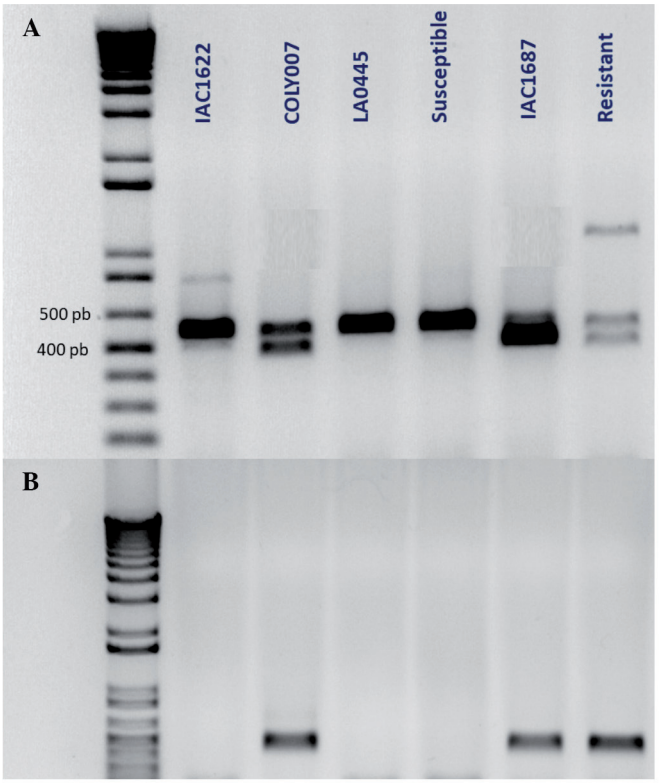
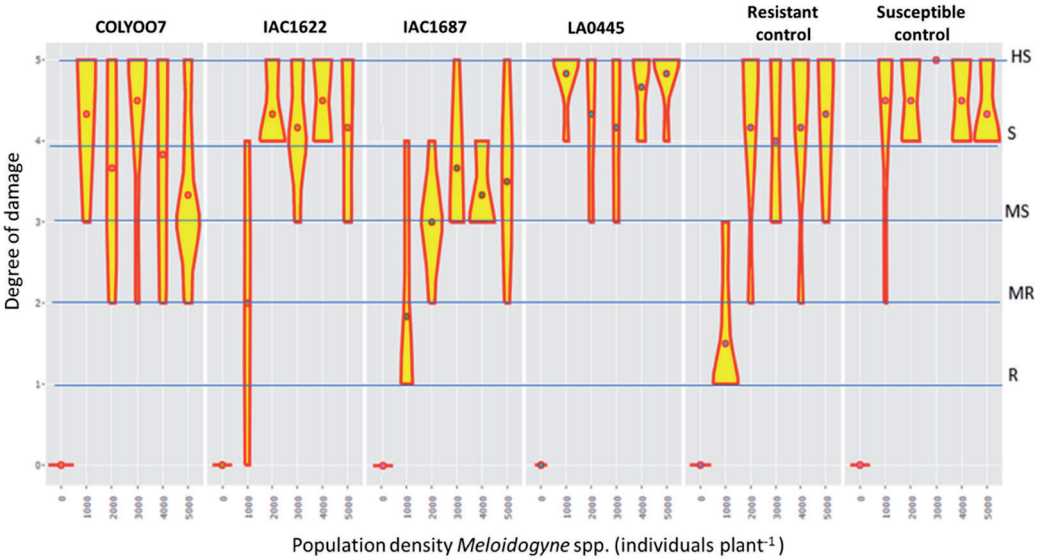


Figure 2. Phenotypic response to different inoculated population densities of *Meloidogyne* spp. Violin plot in R it is noted plant distribution by genotype in the degrees of the damage scale proposed by Taylor and Sasser (1983).



HS: Highly susceptible; S: susceptible; MS: moderately susceptible; MR: moderately resistant; R: resistant.

The higher the density of the inoculated population, the greater the number of individuals recovered. In the total population recovered, the inoculation of 2000 individuals plant^{-1} or more did not show significant differences in all accessions evaluated; in contrast, the values reached for the doses 1000 and 0 individuals plant^{-1} showed significant differences ($p < 0.05$) between IAC1687 and commercial resistant genotype in relation with the others genotypes evaluated. The accession IAC1687 showed a particular behavior indicated by values below 50 000 individuals plant^{-1} in total population recovered of *Meloidogyne* spp. in inoculated population densities of 1000, 2000 and 3000 individuals plant^{-1} (Figure 3).

Genotype-phenotype association

The association of the presence of the *Mi-1* resistance gene and the response to the different population densities of *Meloidogyne* spp., indicated that genotype IAC1687, with the resistance allele of the gene *Mi* (*Mi*/*Mi*) (Figure 1), was classified as moderately resistant in the treatment 1000 individuals plant^{-1} and the lowest general population recovered (Table 3, Figure 2).

The commercial resistant genotype showed a high tolerance level to *M. incognita*, *M. javanica* and *M. arenaria*; however, this genotype is heterozygote (*Mi*/*mi*) (Figure 1) and responded as moderately resistant in the lowest inoculated population density. For this genotype, resistance was broken when higher population densities of the pathogen were inoculated (Table 3, Figure 2). Genotype COLY007, despite being heterozygote (*Mi*/*mi*), was classified as moderately susceptible in all population densities, probably due to the genotype-environment interaction in the phenotypic evaluation conditions.

This demonstrates the importance of associating the presence of resistance genes with their phenotypic response to guarantee the effectiveness of resistance in breeding programs in integrated crop management. The other accessions presented the susceptibility allele (*mi*/*mi*) and were classified as moderately susceptible (Table 3, Figure 2).

Figure 3. Total population recovered in the interaction genotype × inoculated population density of *Meloidogyne* spp. 0, 1000, 2000, 3000, 4000, and 5000 individuals plant^{-1}.

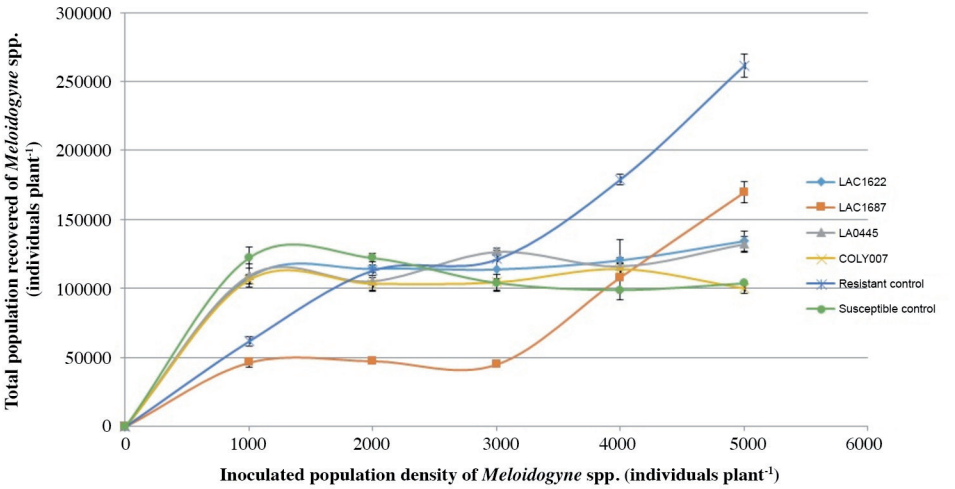


Table 3. Association of the presence of the *Mi* gene in tomato genotypes and their response to *Meloidogyne* spp.

Accession	Resistance Mi-23	Susceptibility Mi-23	Genotype	Resistance classification[1]
COLY007	Yes (*Mi*)	Yes (*mi*)	*Mi*/*mi*	Moderately susceptible
IAC1622	No	Yes	*mi*/*mi*	Moderately susceptible
Susceptible genotype	No	Yes	*mi*/*mi*	Moderately susceptible
LA0445	No	Yes	*mi*/*mi*	Moderately susceptible
IAC1687	Yes	No	*Mi*/*Mi*	Moderately resistant
Resistant genotype	Yes	Yes	*Mi*/*mi*	Moderately susceptible

[1]Classification of the phenotypic response according to Sañudo et al. (2003).

In relation to the total population of *Meloidogyne* spp. recovered, it was found that genotype IAC1687 showed the lowest number of individuals recovered between doses 1000 and 3000 individuals plant^{-1}, reaching values between 45 102 and 46 426 individuals 100 g^{-1} root. The commercial resistant genotype showed a population of 61 965 individuals 100 g^{-1} root at the lowest inoculated dose, which continues to increase as the inoculated pathogen density increases, reaching a population of 262 494 individuals 100 g^{-1} root at 5000 individuals plant^{-1} (Figure 3).

The presence of the resistance allele and the response of the phenotype susceptible to the pathogen may be due to high population densities or virulent populations of the pathogen to the gene *Mi*, characteristics that can breakdown the resistance in the plant (Maleita et al., 2012; Barbary et al., 2015). It has been reported that the heterozygous or homozygous state of the locus *Mi* affects the degree of resistance to *Meloidogyne* species (Kesba et al., 2015; Seid et al., 2015). Genotypes with the heterozygous form (*Mi*/*mi*), as is the case with the commercial resistant genotype, are less resistant than the homozygous genotypes (*Mi*/*Mi*), as observed in accession IAC1687, which presented the resistance allele in dominant homozygosis (*Mi*/*Mi*). These genotypes maintained its classification of resistance to low doses of the pathogen and in the general average of the population densities applied (Table 3). It has been shown in tomato that the gene *Mi* provides partial protection against the development of different species of the genus *Meloidogyne* (Maleita et al., 2012).

The response of the variation in the degree of susceptibility found is due to the differences or genetic composition of the genotypes at different population densities of the pathogen resulting in a genotype-pathogen interaction (Sukhjeet et al., 2019). Several studies report that the selection pressure gives rise to the appearance of virulent biotypes of *M. incognita*, with the ability to reproduce in commercial crops established in the field (Barbary et al., 2015). The differential response of tomato genotypes associated with the allelic form of the gene *Mi* is consistent with that observed in commercial crops in the Colombian coffee region, which have good performance at low densities of the pathogen population. However, with successive crop cycles, nematode population densities increase, drastically reducing yields, generating substantial economic losses, where the response of materials registered as resistant is not evident.

One of the reasons why the resistant commercial control used in the present study had not behaved as highly resistant in any of the population densities evaluated, could be due to an increase in soil temperature to more than 28 °C by periods longer than 3 h, affecting the efficiency of the *Mi-1* gene as has already been reported in other studies (de Carvalho et al., 2015). Temperatures above 28 °C in the soil have been observed in the conditions of the present study (data not shown). Another reason could be due to the presence of virulent *Meloidogyne* biotypes to the *Mi-1* gene that, due to the high use of these commercial materials, creates a selection pressure in the pathogen. However, the results in the present study show that there are significant differences between the resistant control and the commercial susceptible control at the lower population density of the population evaluated 1000 individuals plant^{-1}.

Although there are tomato genotypes with the resistance gene *Mi* to inhibit the attack and damage of *Meloidogyne* spp., this continues to show considerable root nodulations that affect yield and production costs of the different horticultural species (Barbary et al., 2015). The presence of various *Meloidogyne* species (Quénéhervé et al., 2011) has been confirmed in the soil, which can cause damage in plants that present the gene *Mi-1*, which does not affect these nematode species (Pérez-Almeida et al., 2015); this leads to the need of searching for new sources of resistance to this pathogen.

The inoculated population density of 1000 individuals may be optimal for evaluating promising tomato germplasm for its resistance to *Meloidogyne* spp. Genotype IAC1687 showed the best response in the variables of damage evaluated. Thus, it can be suggested as promising in genetic improvement programs due to its genetic potential against *Meloidogyne* spp.

Agronomic variables

The response of tomato genotypes to different population densities of *Meloidogyne* spp. resulted in significant differences ($p \leq 0.05$) in the variables fresh weight of roots (FWR), fresh weight of aerial part of the plant (FWA), dry weight of roots (DWR) and dry weight of aerial part of the plant (DWA) and number of fruits (NF) (Table 4). The variables root and stem lengths and weight of fruits obtained general averages of 45.32 ± 5.59 cm, 168.38 ± 6.86 cm and 31.12 ± 18.65 g, respectively, without significant differences (data not showed).

Khanzada et al. (2012) reported significant reductions in stem length and root weight in tomato plants inoculated with 500 juveniles of the nodulator nematode *M. incognita* in relation to the control (uninoculated). Corrales et al. (1999) inoculated different densities of *Meloidogyne* spp. in *Solanum quitoense* between 2000 and 10 000 eggs plant^{-1}, finding significant negative effects on the evaluated variables. As the applied population density increased, there was an increase in the presence of nodulations, which was reflected in the height of the plant with a reduction from 26% to 47%. The

Table 4. Effect of different population densities of *Meloidogyne* spp. on the behavior of agronomic variables in tomato genotypes.

Variable	0 ind. plant^{-1}	1000 ind. plant^{-1}	2000 ind. plant^{-1}	3000 ind. plant^{-1}	4000 ind. plant^{-1}	5000 ind. plant^{-1}
Fruit number	5.4a	2.5b	2.8b	3.0b	2.5b	3.4b
Fresh weight root, g	31.8c	44.3b	47.2b	52.2ab	61.0a	56.4a
Dry weight root, g	10.1d	9.6d	11.8cd	14.1bc	18.6a	16.0b
Aereal fresh weight, g	124.6ab	112.0b	131.0ab	147.8a	133.5ab	132.1ab
Aereal dry weight, g	25.0ab	22.6b	24.5ab	24.9ab	24.6ab	27.7a

Different letters denote significant differences between genotypes according to Duncan's multiple rank test (α= 0.05).

fresh and dry aerial weight experienced reductions of up to 64% in the highest densities. The variables FWR and DWR presented a gradual increase that is related to inoculum densities and is reflected in the increase of the nematode population in each treatment, which agrees with Corrales et al. (1999), where they report that FWR is greater due to the nodular effect caused by the infection of the pathogen.

Our study at 60 d after inoculation found that the treatment without inoculation of nematodes manifested a greater number of fruits, unlike the other treatments where they presented lower averages, but did not decrease gradually to the doses. This is possibly because when *Meloidogyne* spp. produce galls in roots affects the absorption of water and nutrients and its transport from the roots to the shoots (Rodiuc et al., 2014), which is reflected in the reduction of flowering and consequently in the quality and number of fruits (Dagatti et al., 2014). Additionally, the response of the plant to nematode parasitism causes morphological and physiological changes that affect photosynthetic processes (Strajnar et al., 2012). These effects increase during nematode infection, which are clearly observed in susceptible genotypes (Beyan et al., 2019; Cardoso et al., 2019).

Correlation of variables

The correlation between the damage scale (DS) and the total nematode population recovered (TNPR) was directly proportional ($p < 0.05$), where the highest correlation values were presented in the genotypes classified as moderately susceptible LA0445, COLY007, and susceptible control, with correlations of 0.89, 0.85 and 0.78, respectively, indicating that the greater the damage scale, the greater the total nematode population recovered. In contrast to the genotype classified as moderately resistant, IAC1687 presented a lower correlation of 0.45, which is consistent with the lower values of DS and TNPR in the genotypes evaluated (Table 5).

The relationship between the variables number and weight of fruits and the disease incidence variables (DS and TNPR), showed a differential behavior between genotypes (Table 5). An inverse correlation was found for DS and number of fruits for genotypes IAC1622, COLY007 and the susceptible control with values of -0.48, -0.39 and -0.29, respectively, whereas the damage scale increases, the number of fruits decreases. Similarly, an inverse correlation was found between DS and weight of fruits in genotypes IAC1622, IAC1687 and COLY007, with correlations of -0.48, -0.35 and -0.45, indicating a decrease in the weight of the fruits caused by nodular damage in the roots of the plant. This shows the negative impact

Table 5. Pearson correlation between damage incidence and plant development variables: Damage scale (DS), total population of nematodes recovered (TPNR), number of fruits (NF), fruit weight (FW), fresh weight of roots (FWR), fresh weight of the aerial part of the plant (FWA), dry weight of roots (DWR) and dry weight aerial part of the plant (DWA).

	Genotypes					
Variables	IAC1622	IAC1687	LA0445	Resistant	Susceptible	COLY007
DS-NF	-0.48857**	NA	NA	NA	-0.29623	-0.39814
DS-FW	-0.48542	-0.35403	NA	NA	NA	-0.45275
DS-FWR	0.34433	NA	0.47531	0.39047	NA	NA
DS-DWR	NA	NA	0.42459		NA	NA
DS-DWA	NA	NA	NA	-0.35280	NA	NA
DS-TPNR	0.69447	0.45112	0.89092	0.64776	0.85100	0.77980
TPNR-NF	-0.59557	-0.36944	NA	NA	-0.33479	-0.39695
TPNR-PF	-0.53453	-0.36579	NA	NA	NA	NA
TPNR-FWR	NA	NA	NA	0.54649	NA	0.39765
TPNR-DWR	NA	NA	NA	0.30414	NA	0.46348

**Pearson correlation values, only correlations with values of $p < 0.05$ are presented.
NA: No correlation was observed between variables ($p < 0.05$).

of the density of pathogens on the variables of the yield components that drastically affect crop yield at densities greater than 1000 individuals plant^{-1} (Table 5). Salazar-Antón and Guzmán-Hernández (2013) evaluated the effect of the initial density (Pi) against the final density (Pf) of *Meloidogyne* spp. in the development and yield of tomato, finding an inverse correlation between Pi and yield of tomato, a Pi greater than 620 nematodes 100 g^{-1} soil reduced the yield to less than 1.02 kg fruit plant^{-1}. Cervantes-Moreno et al. (2014), in their study of 26 genotypes native of Mexico tolerant to *M. incognita*, applied 100 000 eggs-juveniles plant^{-1}, finding fruits of smaller diameter, decrease in plant height and length of roots.

The results obtained showed that the inoculated population density of *Meloidogyne* spp. affected the expression and efficacy of the *Mi-1* gene to maintain resistance to densities greater than 1000 individuals, where the increase in pathogen densities had a negative effect on the number of fruits which affects the productive potential of the plant. This agrees with Sharma and Sharma (2015), who observed that the increase of the intensity of the infection by nematodes of the radical node (500, 750, 1000, 1250 and 1500 juvenile stage 2, J2) negatively affected the growth of the plant and biomass. Maleita et al. (2012) reported that increased inoculation densities (2500, 5000 and 10 000 eggs + J2s) of *M. javanica* and *M. hispanica* increased the number of galls or nodulations in resistant tomato plants.

The most discriminating variables that allowed determining the inoculated population density to evaluate the differential response of tomato germplasm to *Meloidogyne* spp. were FWR and DWR, total population of nematodes recovered and damage scale. The dose of 1000 individuals or less may be optimal to evaluate tomato germplasm promising for its resistance to *Meloidogyne* spp. and maintain this condition during plant development.

The IAC1687 genotype showed the best response in the evaluated damage variables, so it can be included as promising for its genetic potential of resistance to *Meloidogyne* spp. in genetic improvement programs and as rootstocks of commercial tomato cultivated as a sustainable and efficient alternative in integrated management programs of radical nodulation disease in tomato crops.

CONCLUSIONS

Densities greater than 1000 individuals plant^{-1} can break resistance, leaving aside promising genotypes for future integrated pathogen management programs including genetic resistance. Additionally, it is necessary to correlate the genotypic and phenotypic responses to guarantee the success of the selection supported by molecular markers such as SCAR Mi-23.

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