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RESEARCH ARTICLE

Antagonist effect of native bacteria of the genus *Bacillus* on the root-knot nematode (*Meloidogyne* spp.) in tomato germplasm

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ABSTRACT

The root-knot nematode (*Meloidogyne* spp.) causes losses of up to 68% of tomato (*Solanum lycopersicum* L.) production, management practices are limited and toxic chemicals are mostly used. An integrated management alternative was evaluated based on the antagonist effect of native bacteria of the genus *Bacillus* on the nematode using four tomato germplasms, two commercial (susceptible and tolerant) and two wild (IAC 1687 and COLY 007). In the in vitro phase, strains *B. infantis* (GIBI 177), *B. altitudinis* (GIBI 187), *B. pumilus* (GIBI 195), *B. amyloliquefaciens* (GIBI 200) and *B. pumilus* (GIBI 206) were evaluated and the percentage of inhibition of nematode egg hatching at 9 d after inoculation and the percentage of mortality in juveniles at 72 h after incubation were determined. The results showed that all native strains presented a control effect under controlled conditions, but *B. infantis* being the bacterium with the highest selection index (0.65). In the field phase, a split-plot design was established, the main plot being the four-tomato genotype and the secondary plot the bacillus strain, and the percentage of severity, number of eggs and juveniles were evaluated. The tomato genotype with the best response to *Meloidogyne* spp. attack was IAC 1687, when obtaining values of 2088.8 ± 599.1 individuals 100 g^{-1} root. The bacterium that presented the best interaction with 3 of the 4 genotypes evaluated was *B. pumilus* (GIBI 206), in IAC 1687 with 1731.33 ± 1382. 57 individuals 100 g^{-1} root, in the resistant genotype with 16627 ± 4588.1 individuals 100 g^{-1} root and in the susceptible genotype with 3303.83 ± 1256.25 individuals 100 g^{-1} root. This study reports the use of tomato genetic resources and antagonist bacteria as a potential integrated management of the root-knot nematode.

Key words: Biological control, genotypes, inhibition, severity, *Solanum lycopersicum*.

INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is considered one of the vegetables with the largest area planted worldwide, being one of the most consumed foods, both fresh and processed products. Its production in 2019 reached 180 million tons in an area of 5.0 million hectares harvested worldwide (Faostat, 2021).

One of the main limiting factors in the crop are the phytoparasitic nematodes of the genus *Meloidogyne* spp. The species that cause the greatest losses are *M. javanica*, *M. hapla* and *M. arenaria*, limiting production with estimated losses between 28% and 68% (Salazar-Antón and Guzmán-Hernández, 2013). Symptoms associated with the root-knot nematode are identified by the formation of small protuberances, nodules, or galls on the roots, which limit the transport and movement of water and nutrients to the plant, and as a consequence its productive capacity is reduced (Kepenekci and Saglam, 2018). Subsequently, symptoms appear as delayed development and weakening of the plant, yellowing in older leaves and a considerable reduction in production, affecting both the quantity and quality of fruits (Ortiz et al., 2015).

In tomato cultivation, preventive and curative practices such as physical treatments to the soil (solarization, flooding), crop rotation, application of organic amendments, application of chemicals that are highly toxic, biological control, resistant varieties and genetic control are carried out (Huang et al., 2018); initially, this genetic resistance to attack by species such as *M. incognita*, *M. javanica* and *M. arenaria*, was found in wild species of *Lycopersicon peruvianum* (L.) Mill., through the rapid production of reactive oxygen species (ROS) (Kadota et al., 2015), this resistance to root-knot nematodes in tomatoes is characterized by the presence of the *Mi* gene associated with a hypersensitivity reaction, which causes histological changes in the root (Williamson and Roberts, 2009). The use of genetic resistance is a highly efficient way to control the disease and has a positive impact in ecological and economic terms (Cardoso et al., 2019). Currently, many commercial materials are available with resistance to distinct species of *Meloidogyne* spp.; however, after establishing them in commercial crops, there is evidence of disease attack causing significant economic losses, making production systems unsustainable over time.

Other promising alternatives for the management of the root-knot nematode is biological control, some *Bacillus* spp. have been found to have a positive effect on plant growth and include biocontrol mechanisms such as antibiosis, competition for space and available nutrients, and induction of resistance (Podile and Kishore, 2007). Several studies demonstrate the potential of *Bacillus* species for the control of *Meloidogyne* spp. populations in tomato crop, including *B. cereus* (Hu et al., 2017), *B. firmus* (d'Errico et al., 2019), *B. subtilis* (Liu et al., 2014), and *B. megaterium* (Huang et al., 2009), among others, which inhibit hatching of juveniles and infection by second instar juveniles to plants, by mechanisms such as production of volatile organic compounds (Huang et al., 2009), production of hydrolytic enzymes such as chitinases and proteases (Lee and Kim, 2015) and extracellular substances such as sphingosine or through induced systemic resistance (ISR) in plants (Gao et al., 2016).

In the present study, promising *Bacillus* spp. strains were selected for the control of *Meloidogyne* spp. under in vitro conditions and the effects of genotype-bacteria interaction were determined for the control of the phytoparasitic nematode as an integrated management option taking advantage of the use of tomato genetic resources, in order to project the incorporation of strategies that can be included in the integrated management of the disease and of the crop in search of more sustainable production systems.

MATERIALS AND METHODS

Native and commercial control bacteria used in vitro evaluations

Bacillus infantis isolates (GIBI 177 accession KX965619), *B. altitudinis* (GIBI 187 accession KX965627), *B. pumilus* (GIBI 195 accession KX965634), *B. amyloliquefaciens* (GIBI 200 accession KX965638) and *B. pumilus* (GIBI 206 accession KX965644) were supplied by the Collection of Microorganisms of the Catholic University of Manizales (CMUCM), initially isolated from lignocellulosic residues of higuerilla or castor-bean (*Ricinus communis* L.) from the Department of Caldas, Colombia, which were later morphologically, biochemically and molecularly characterized. These isolates were preserved in 30% glycerol at -80 °C, for reactivation, the strains were seeded by depletion in LB agar (Luria Bertani) and incubated at 28 °C for 24 h. The isolated colonies, were inoculated in LB nutrient broth and put to grow in agitation at 110 rpm at 30 °C for the times required for each species, adjusting them to a concentration of 1×10^8 CFU mL^{-1} (Cabra Cendales et al., 2017). The commercial control (TCOM) has a concentration of *B. subtilis*, race QTS 713 of 1×10^9 UFC g^{-1} and the minimum dose recommended in the technical data sheet (2.5 mL in 1 L water) was prepared.

Obtaining eggs and juveniles of *Meloidogyne* spp.

The inoculum was obtained from tomato plants sown at the experimental farm "Montelindo" of the University of Caldas, located in the municipality of Santagueda-Caldas (5°05' N, 75°40' W; 1050 m a.s.l.), 22.5 °C mean temperature, 76% relative humidity, 2100 mm annual rainfall and 2010 h annual sunshine.

The extraction of *Meloidogyne* spp. eggs was conducted following the methodology described by Hussey and Barker (1973). Eggs were then selected with a micropipette using a microscope (CX31, Olympus,

Tokyo, Japan) with a 4X objective and adjusted to a concentration of 200 eggs in 2.5 mL sterile distilled water for each experimental unit. To obtain second-stage juveniles (J2) of *Meloidogyne* spp. eggs were placed in a modified Baermann funnel (Castillo et al., 2013) and incubated at 31 °C for 2 to 4 d (Xiang et al., 2017). The J2 were selected with micropipette using an CX31 brand microscope at 4X objective and adjusted to a concentration of 40 J2 in 2.5 mL sterile distilled water, per Petri dish as experimental unit.

Inhibition of egg hatching and percentage mortality in *Meloidogyne* spp. juveniles

A completely randomized design (CAA) was used with five treatments consisting of the isolates *Bacillus infantis* (GIBI 177), *B. altitudinis* (GIBI 187), *B. pumilus* (GIBI 195), *B. amyloliquefaciens* (GIBI 200), and *B. pumilus* (GIBI 206). To determine the effect of native bacteria on *Meloidogyne* spp. eggs, the percentage inhibition of hatching at 9 d of incubation (ddi) was calculated, and five replicates of each treatment were established. In juveniles, the number of dead individuals (% mortality) was counted after 72 h of incubation (hdi), and five replicates were also established for each treatment. The data from each experimental unit were corrected for the percentage of inhibition in egg hatching and the percentage of natural mortality of juvenile *Meloidogyne* spp. in the control treatment, using the formula of Schneider-Orelli (1947) cited by Leguizamón and Padilla (2001).

The nematostatic effect was determined on the nematodes that were in contact for 24 h with the bacteria evaluated, then transferring them to sterile distilled water to evaluate the percentage of mobility during the following 24 h. A completely randomized design with five replicates per treatment was established.

Statistical analysis

An ANOVA was performed to evaluate significant differences between treatments ($p < 0.01$) for the variables of percentage inhibition of egg hatching, percentage mortality of juveniles and nematostatic effect, then Tukey's comparative tests were calculated for differences in the means of each treatment ($p \leq 0.05$), using the SAS 9.1 statistical program (SAS Institute, Cary, North Carolina, USA).

Selection index

A selection index considering the effect on eggs and juveniles of *Meloidogyne* spp. was determined by applying the following formula:

$$\text{Selection index} = (0.5)\frac{(\%\ \text{Treat mort.} - \text{Prom.}\ \%\ \ \text{Total mort.})}{\text{Standard deviation}\ \%\ \text{Treat mort}} + (0.5)\frac{(\%\ \text{Inh. treat hatch} - \text{Prom.}\ \%\ \text{Total hatch inh})}{\text{Standard deviation}\ \%\ \text{hatch Inh.}}$$

where Treat. mort. is treatment mortality, Total mort. is total mortality, Inh. treat hatch. is inhibition of treatment hatching, and Total hatch. inh. is Total hatching inhibition.

The results with values higher than zero indicate a positive control effect on nematodes, the higher the value obtained, the better the effectiveness of the strain, for this reason the bacteria that obtained the three highest values in selection index were chosen to be evaluated later in experimental plots in the field phase.

Tomato genotypes evaluated in the field

Two wild tomato genotypes were evaluated, IAC 1687 from the Brazilian subcollection (*Solanum lycopersicum* L. var. *cerasiforme* (Alef.) Voss) and COLY 007 (*S. lycopersicum* var. *cerasiforme*) from Colombia, and two commercial controls, one susceptible and one resistant to *Meloidogyne* spp. according to the commercial data sheet. Germination of the genotypes was carried out at the Tesorito farm, in the Municipality of Manizales (Caldas, Colombia; 5°01'49" N, 75°26'13" W; 2340 m a.s.l.), average temperature 17 °C, annual rainfall 1800 mm, relative humidity 78% and annual sunshine 1215 h. Trays of 72 locules and a "Sphagnum" peat substrate were used. Transplanting was conducted after 30 d at the Montelindo farm, under a macro-tunnel greenhouse system, to guarantee controlled conditions during the experiment. The plants were distributed in individual troughs per experimental unit with a 1.2 caliber "V" plastic overstory. The soil used for planting was disinfected with dazomet (3,5-dimethyl-1,3,5-thiadiazinane-2-thione, $C_5H_{10}N_2S_2$, BASF SE, Ludwigshafen, Germany), at a

dose of 500 kg ha^{-1}, and black plastic mulch was used for 30 d as a solarization treatment. Subsequent laboratory analyses were conducted to corroborate the biological vacuum in the soil. Drip irrigation was applied according to the water demand of the plants at each stage of development to prevent the spread of the pathogen.

Treatments evaluated in the field

The strains of *Bacillus infantis* (GIBI 177), *B. amyloliquefaciens* (GIBI 200) and *B. pumilus* (GIBI 206), selected for their biocontrol potential, were standardized at a concentration of 1×10^8 CFU mL^{-1} and a dose of 5 mL L^{-1} water was used, applying a mixture volume of 200 mL $plant^{-1}$, three applications were made in drench, at intervals of 20 d; the first week after transplanting the seedlings, the second one 27 d after transplanting (ddt) and the third one 47 ddt. The commercial control (TCOM) was applied at the same time as the native bacteria, following the recommendation of the commercial company, and using an average dose of 5 mL L^{-1} water. The chemical insecticide and nematicide cadusafos (2-[butan-2-ylsulfanyl(ethoxy)phosphoryl]sulfanylbutane, $C_{10}H_{23}O_2PS_2$, FMC CORPORATION, Philadelphia, Pennsylvania, USA) was used at 5 g per plant according to the manufacturer's recommendation.

Pathogen inoculation was performed with a suspension of nematodes at a concentration of 1000 individuals in 200 mL water for each plant, for the application holes of approximately 5 cm depth were made in four cardinal points of the plant and 50 mL suspension were applied (Cardona-Piedrahita et al., 2016; You et al., 2018).

Field experimental design under controlled conditions

A split-plot design was established, where the main plot was constituted by four tomato genotypes and the secondary plot by three *Bacillus* spp. strains, commercial control *B. subtilis* race QTS 713 (TCOM), chemical product cadusafos, control with nematodes (TNEM), and the control without nematodes (TSNEM), in an arrangement of three blocks inside each main plot with six plants per treatment per block, being the plant the experimental unit for a total of 18 plants per combination between the genotype and the bacteria in the presence of *Meloidogyne* spp.

Variables evaluated

Destructive sampling was done when the plants reached 60 d after transplanting and the total nematode population (TPN) was evaluated by quantifying the number of individuals (eggs and juveniles) in 100 g root by optical microscopy (CX31, Olympus, Tokyo, Japan)) (40X) and the damage scale (DS) using the severity scale proposed by Taylor and Sasser (1983) (Table 1).

Table 1. Damage scale to evaluate the phenotypic response in tomato plants against *Meloidogyne* spp. [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).

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

Statistical analysis

Using SAS 9.1 statistical software, ANOVA type mean tests were performed to determine significant differences between treatments ($p \leq 0.05$), then Tukey type mean tests were performed ($p \leq 0.05$) in order to find the most promising alternatives for the control of *Meloidogyne* spp. that can be included in an integrated management of the disease and of the tomato crop.

RESULTS

Percentage inhibition on hatching of *Meloidogyne* spp. eggs in vitro

All strains presented antagonist effects on *Meloidogyne* spp. eggs, according to ANOVA, being significantly different ($p \leq 0.05$). The most effective treatments for the control of root-knot nematode (*Meloidogyne* spp.) eggs under in vitro conditions were those corresponding to *B. infantis* (GIBI 177), *B. pumilus* (GIBI 206) and *B. amyloliquefaciens* (GIBI 200) isolates, with egg hatch inhibition percentages of 32.14 ± 10.8%, 30.8 ± 9.4% and 29.23 ± 8.4%, respectively (Table 2).

Table 2. Effect of native bacteria of the genus *Bacillus* on eggs and juveniles of *Meloidogyne* spp. Different letters indicate significant differences ($p \leq 0.05$) between bacteria for each variable analyzed, using Tukey comparative tests. Values of ± indicate the standard deviation of each treatment for the variable analyzed. *Isolates with better performance for taking to the field. Values closer to 1 indicate better control. TCOM: Commercial control; ddi: days of incubation; J2: second-stage juveniles of *Meloidogyne* spp.

Treatment	Inhibition percentage (9 ddi)	Mortality percentage in J2 (72 h)	Nematostatic effect	Selection index
GIBI177 *B. infantis*	32.14 ± 10.8^{a}	61.16 ± 0.0^{a}	75.96 ± 5.0^{c}	0.65*
GIBI206 *B. pumilus*	30.80 ± 9.4^{a}	61.16 ± 0.0^{a}	96.66 ± 4.0^{e}	0.59*
GIBI200 *B. amyloliquefaciens*	29.23 ± 8.4^{a}	61.16 ± 0.0^{a}	54.19 ± 8.0^{b}	0.52*
GIBI195 *B. pumilus*	28.15 ± 8.5^{a}	61.16 ± 0.0^{a}	63.46 ± 13.2^{c}	0.47
GIBI187 *B. altitudinis*	25.85 ± 8.5^{a}	61.16 ± 0.0^{a}	89.12 ± 4.4^{d}	0.37
TCOM	23.05 ± 9.9^{a}	1.66 ± 5.1^{b}	0.0 ± 0.0^{a}	-0.77

Percentage mortality in juveniles of *Meloidogyne* spp. in vitro

The strains evaluated presented significant differences according to ANOVA, in the percentage of mortality ($p \leq 0.05$). In all cases, when the juveniles came into contact with the native bacteria 72 h after inoculation (hdi), they presented a corrected mortality effect of 61.16 ± 0%. The commercial control (TCOM) was the only treatment that did not kill juveniles and remained at the same level as the control treatment (Table 2).

The ANOVA of the nematostatic effect showed significant differences ($p \leq 0.05$). The bacterium with the highest nematostatic effect was *B. pumilus* (GIBI 206) as 96.66 ± 4% of juveniles remained immotile, followed by *B. altitudinis* (GIBI 187) with 89.12 ± 4.4% of juveniles immotile (Table 2).

Selection of bacteria for control of *Meloidogyne* spp. in vitro

In the in vitro phase (controlled conditions), *B. infantis* (GIBI 177) was the bacterium with the highest selection index (0.65), followed by *B. pumilus* (GIBI 206) and B. *amyloliquefaciens* (GIBI 200) with values of 0.59 and 0.52, respectively (Table 2). So, they were selected to be evaluated in the field to know their behavior in ex situ conditions closer to those of tomato producers in the department of Caldas, Colombia.

Analysis of tomato genotypes in the presence of *Meloidogyne* spp. in the field

The ANOVA of the effect of genotypes on the damage generated by nematodes showed significant differences ($p \leq 0.05$) for the variables analyzed. The wild genotypes behaved as promising to attack these phytoparasites and presented exceptionally low values with respect to the commercial ones, especially genotype IAC1687 when obtaining values of 2088.8 ± 599.1 individuals 100 g^{-1} root. After the wild genotype IAC 1687, the COLY007 genotype obtained the best results against nematode

control, positioning the wild genotypes as an object of study to generate potential alternatives that can be included in genetic improvement programs. The number of nematodes and the percentage of severity in the plant was significantly higher in the resistant genotype that presented mean values of 22092.8 ± 3028.3 individuals 100 g^{-1} root and severities of 5 ± 4.8%. In the other genotypes the percentage remained below 2.5% of root area affectation, which according to the Taylor and Sasser scale (Taylor and Sasser, 1983), is in grade 1 (G1), indicating a resistant phenotypic response of the genotypes evaluated (Figure 1).

Analysis by treatment

All native bacteria presented juvenile values below the control with nematodes (14292.5 ± 4665.7 individuals 100 g^{-1} root), in the statistical analysis (ANOVA) there were significant differences between them ($p \leq 0.05$). *B. pumilus* (GIBI 206) and TCOM presented values of 6592.7 ± 2314.4 and 6930.4 ± 2783.4 individuals 100 g^{-1} root respectively, positioning themselves as the most promising for the control of *Meloidogyne* spp., followed by chemical control with cadusafos and other native bacteria; all alternatives mitigated the presence of individuals compared to the control with nematodes (Figure 1). These results also corroborate what was observed in the in vitro phase, where an inhibition in egg hatching and egg damage due to the effect of these bacteria was evidenced. *B. pumilus* (GIBI 206) was the treatment with the lowest severity percentage (1.08 ± 1.39%), although the other treatments with native bacteria also presented severity percentages below the chemical product, which obtained values of 4.83 ± 5.81% (Figure 2).

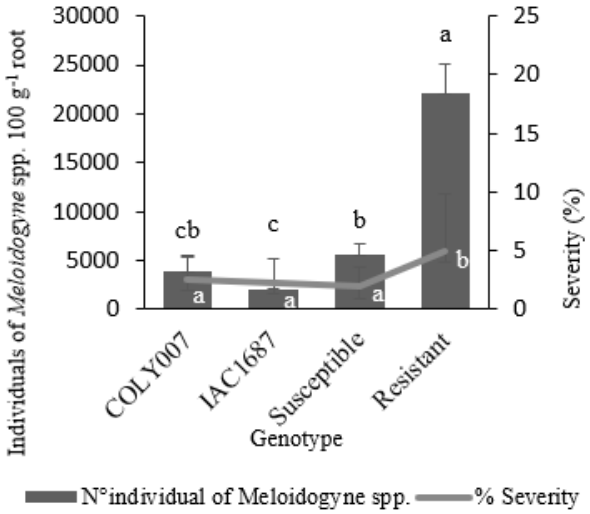

Figure 1. Effect of tomato genotypes on the number of individuals in 100 g root and percentage of severity of *Meloidogyne* spp. Different letters indicate significant differences ($p \leq 0.05$) for each genotype, by Tukey comparative tests. Bars indicate the standard deviation of each genotype analyzed.

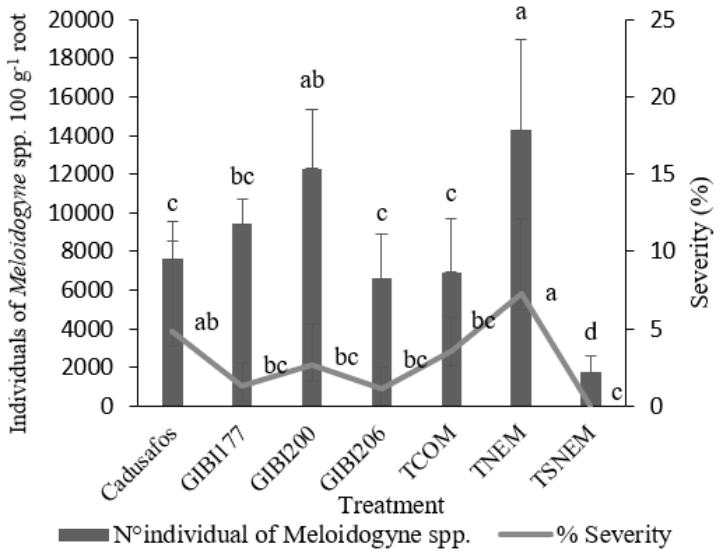

Figure 2. Effect of treatments on the number of individuals in 100 g root and percentage of severity of *Meloidogyne* spp. Different letters indicate significant differences ($p \leq 0.05$) by Tukey comparative tests. The bars indicate the standard deviation of each treatment analyzed. TCOM: Commercial control *Bacillus subtilis* race QTS 713; TNEM: control with nematodes; TSNEM: control without nematodes.

Plant-bacteria interaction

In genotype IAC 1687 all treatments maintained a population of *Meloidogyne* spp. below the control with nematodes (6252.01±2506.77 individuals 100 g^{-1} root), being *B. infantis* (GIBI 177) with 1637.31 ± 1228.70 individuals 100 g^{-1} root and *B. pumilus* (GIBI 206) with 1731.33 ± 1382. 57 individuals 100 g^{-1} root, the native bacteria that obtained a control similar to that of the chemical cadusafos and TCOM; in the variable percentage of severity, *B. pumilus* (GIBI 206) and the chemical cadusafos were the treatments with the lowest percentage of nodulations in the root, both remaining below 1% severity (Figure 3a). In genotype COLY 007 there were also significant differences between the treatments compared to the control with nematodes, the TCOM obtained infestation values of 980.13 ± 451. 43 individuals 100 g^{-1} root very similar to the control without nematodes and it was the bacterium *B. infantis* (GIBI 177) that decreased the population of *Meloidogyne* spp. to a higher level than the other native strains, all treatments had a positive effect on the percentage of severity when compared to the control with nematodes that had a percentage of severity of 13 ± 7% (Figure 3b).

In the resistant genotype, the native strain *B. pumilus* (GIBI 206) presented values of 16627 ± 4588.1 individuals 100 g^{-1} root, remarkably similar to the chemical control cadusafos (15329 ± 4904.55 individuals 100 g^{-1}) exerting a significantly high control effect compared to the control with nematodes that obtained 29784.77 ± 8433. 88 individuals 100 g^{-1} root, against the severity variable analyzed, the native bacteria were the ones that decreased the percentage of nodulations in the roots the most, being *B. infantis* (GIBI 177) with 1.33 ± 1.03% the closest to the values of the control without nematodes that did not present any affectation (Figure 3c). In the susceptible genotype, the native bacteria *B. infantis* (GIBI 177) with 2296.61 ± 1232.14 individuals 100 g^{-1} root and *B. pumilus* (GIBI 206) with 3303.83 ± 1256.25 individuals 100 g^{-1} root were the treatments that presented the lowest nematode population, although there were nonsignificant differences in the effect of the bacteria within this genotype compared to the percentage of severity (Figure 3d).

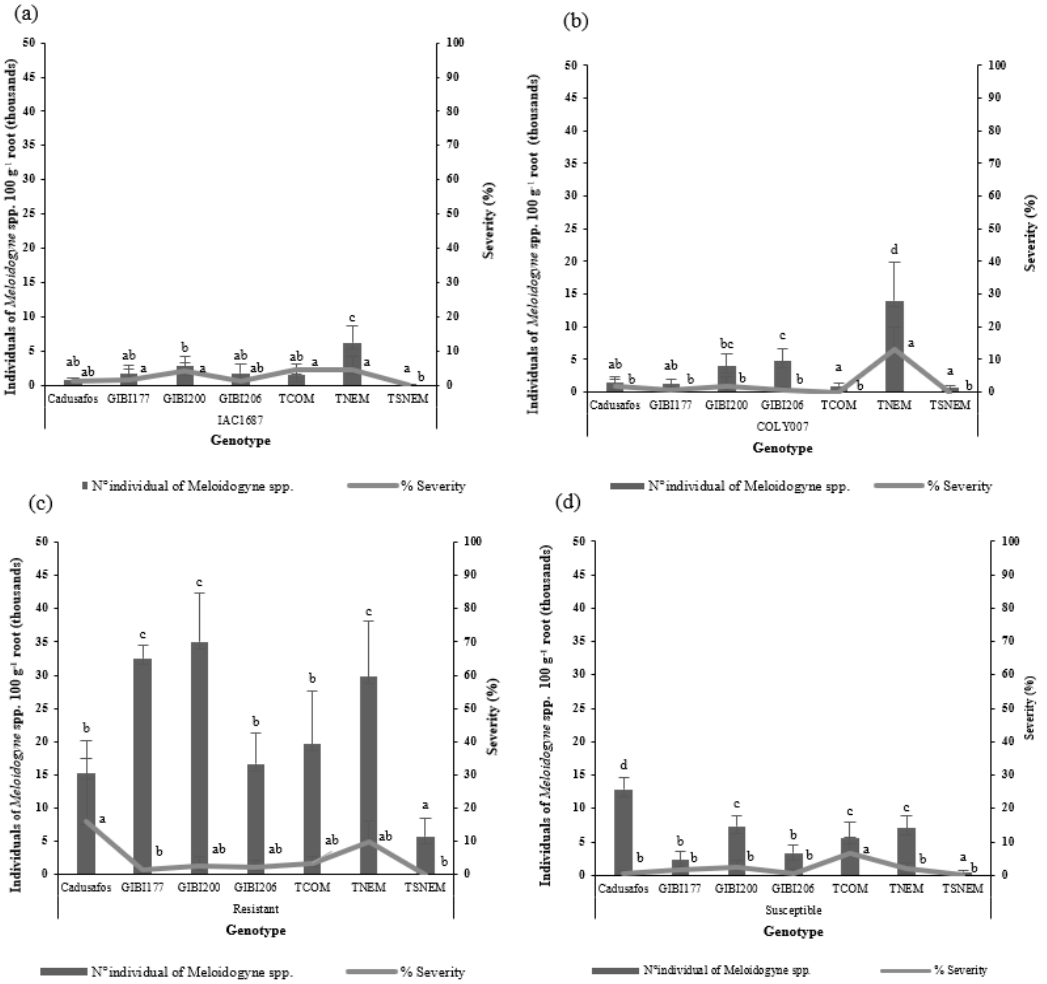

Figure 3. Genotype×Treatment interaction *vs.* the variables of number of individuals in 100 g root and percentage of severity of *Meloidogyne* spp. (a) Effect of treatments on genotype IAC1687. (b) Effect of treatments on genotype COLY007. (c) Effect of treatments on the resistant genotype. (d) Effect of treatments on the susceptible genotype. Different letters indicate significant differences ($p \leq 0.05$) by Tukey comparative tests. Bars indicate the standard deviation of each treatment on the genotype analyzed. TCOM: Commercial control *Bacillus subtilis*, race QTS 713; TNEM: control with nematodes; TSNEM: control without nematodes.

DISCUSSION

Percentage inhibition of hatching of *Meloidogyne* spp. eggs in vitro

There were different effects on the eggs such as necrosis, agglomerates and the formation of biofilm that trapped the eggs and hatched juveniles, in addition, there was evidence of smaller size, poor filling and delayed development since no stages close to hatching were observed, so the eggs may be generating a state of facultative diapause initiated by an exogenous stimulus (Perry et al., 2009) such as the presence of bacteria.

The antagonistic effects of the different *Bacillus* strains on *Meloidogyne* spp. eggs presented in this work confirm those presented by Lee and Kim (2015), demonstrating that *B. pumilus* causes lysis of the egg cuticular layer of *M. incognita* and affects the egg structure of *M. arenaria*, which inhibits hatching because *B. pumilus* L1 is a strong producer of hydrolytic enzymes such as chitinase and protease, which may be

partially involved in nematode suppression, or have cytotoxic effects. Proteases from *Bacillus* spp. could serve as important nematicidal factors for the control of nematode populations in different crops since the cuticle of root-knot nematodes is mainly composed of protein membranes (Perry et al., 2009).

Percent mortality and nematostatic effect on juveniles of *Meloidogyne* spp. in vitro

The mortality effect on *Meloidogyne* juveniles generated by bacteria of the genus *Bacillus* has also been studied by authors such as Hu et al. (2017), who isolated 12 strains of endophytic bacteria from plant tissues and found that three of them, *B. cereus* BCM2, *B. cereus* SZ5 and *B. altitudinis* CCM7 exhibited nematicidal activity against *M. incognita*, as the effect on mortality rate was higher than 90% in all three cases. Zong et al. (2014) identified that *B. cereus* strain S2 can produce some extracellular substances such as sphingosine, which induces the production of ROS in the intestinal tract of treated nematodes that can induce oxidative damage, apoptosis, and cell necrosis in vitro and in vivo.

Several authors have reported the biocontrol effect on *Meloidogyne* spp. from native strains of the genus *Bacillu*s spp. Some suggest that this action may be due to extracellular hydrolytic enzymes produced by *B. pumilus*, which can potentially damage the external structures of juveniles and eggshells (Lee and Kim, 2015). On the other hand, Xiong et al. (2015) proposed that the nematicidal activity of *B. firmus* strain YBf-10 was likely attributed to secondary metabolites rather than proteins and that the various nematicidal activities give *B. firmus* YBf-10 a great advantage for biological control of nematodes since juvenile survival, egg hatching and motility are important physical behaviors critical to the life cycle of the pathogen (Ediz and Dickerson, 1976), therefore, it could interrupt its life cycle and effectively control its reproduction and damage to the host plant.

Analysis of tomato genotypes in the presence of *Meloidogyne* spp. in the field

The highest values of *Meloidogyne* spp. individuals can be evidenced in commercial genotypes, these differences between genotypes may be due to the resistance genes present in them, wild species tend to conserve more of these characters (Bergougnoux, 2014). The heterozygous or homozygous status of the *Mi* locus has been reported to affect the degree of resistance to *Meloidogyne* species (Kesba et al., 2015; Seid et al., 2015). Padilla-Hurtado et al. (2022) corroborated the presence of the *Mi-1* resistance gene in these genotypes evaluated and indicated that genotypes with the heterozygous form (*Mi/mi*), as is the case of the commercial resistant genotype, are less resistant than homozygous genotypes (*Mi/Mi*), such as accession IAC1687, which presented the resistance allele in the dominant homozygosis (*Mi/Mi*). This demonstrates the importance of the genotype and its resistance genes in the field response of plants to the root-knot nematode, suggesting a promising strategy for integrated crop management programs.

Analysis by treatment

In field conditions for the health variables evaluated, all treatments based on native bacteria presented values below the control with nematodes, in addition to presenting severity percentages in some cases similar or lower than those of the chemical product, reaffirming its potential for use as a native biocontroller. Values like these have been reported by Gao et al. (2016) in pot experiments, who infested tomato seedlings with approximately 2000 J2 of *M. incognita*, each plant was irrigated with 25 mL *B. cereus* culture at 20, 40 and 60 d after transplanting and the evaluation was performed at 90 d, by calculating the disease index and control efficiency of the experiment, they showed that *M. incognita* could infect and form large root galls in the control group, while only smaller and smaller galls were formed on the tomato root after treatment with *B. cereus* strain S2.

The observed control of root-root-knot nematodes by native bacteria of the genus *Bacillus* spp. may reflect the direct effects of enzymes and/or compounds that cause inhibition of hatching and mortality in juvenile stages (Lee and Kim, 2015). Numerous species in the genus *Bacillus* have been noted for their potential for incorporation in the control of plant pathogens, as it has been shown that they can reduce disease severity (Adam et al., 2014) and promote plant growth. These microorganisms affect nematode populations directly through the synthesis of hydrolytic enzymes such as chitinases and proteases (Lee and

Kim, 2015), secondary metabolites (Hashem and Abo-Elyousr, 2011), competition for space and nutrients (Beneduzi et al., 2012) or indirectly by generating the induction of systemic resistance in plants (Adam et al., 2014).

Genotype-treatment interaction

The analyses showed that there is an interaction between the different native strains of *Bacillus* spp. and the wild and commercial tomato genotypes present in the region. In addition, it was observed that these interactions differ between each genotype, and vary depending on the strain evaluated. However, one of the limitations observed during the research was related to the sampling of nematode populations. Field observations indicated that nematodes have a clustering habit in certain areas, which can increase data variability. In their study, Bavaresco et al. (2021) found that the AP-3 strain of *B. subtilis* was capable of colonizing both susceptible and resistant cultivars' roots. However, the strain's growth was more efficient in susceptible tomato plants due to its higher similarity with the inoculated strain, which allowed for better attraction and maintenance of the inoculant compared to the resistant genotype. This finding may explain the study's results, in which the susceptible genotype presented lower infestation and severity values than the resistant genotype, despite having this connotation. The formation of microbial communities in the rhizosphere can be specific according to the root exudates of each plant species or even cultivars within a species, as reported by Kawasaki et al. (2016). Furthermore, different strains of *Bacillus* spp. have been reported to mitigate parasitism damage by releasing substances into the rhizosphere capable of obtaining nutrients from the soil, stimulating phytohormone production, and activating systemic resistance in plants (Adam et al., 2014; Hashem et al., 2019).

Induced systemic resistance (ISR) could explain one of the main mechanisms of action of native bacteria of the genus *Bacillus* in the control of the plant-parasitic nematode, because they activate and enhance the activity of biomolecules and enzymes related to plant defense against the pathogen (Sikora et al., 2007). Gao et al. (2016) investigated about the activities of *B. cereus* enzymes related to tomato plant defense to *M. incognita* and after inoculation with the bacterial strain, they increased the activities of phenylalanine ammonia lyase (PAL), peroxidase (POD) and polyphenol oxidase (PPO), furthermore, after liquid chromatography-mass spectrometry (LC-MS) analysis, they identified sphingosine as the main nematicidal substance of *B. cereus*.

There are several mechanisms that can intervene in the defense of *Bacillus* spp. against the root-knot nematode and as shown in this study, the interaction between the different strains according to the genotype established in the field is important, since this will be decisive when designing the control strategies that best adapt to each production system.

CONCLUSIONS

All native strains showed a control effect on *Meloidogyne* spp. in the in vitro phase, being the most promising *Bacillus infantis* (GIBI 177), followed by *B. pumilus* (GIBI 206) and *B. amyloliquefaciens* (GIBI 200).

In all cases, the native bacteria of the genus *Bacillus* presented severity percentages similar to those of the chemical nematicide, reaffirming their potential for use as a native biocontroller, responses that can be synergistic and improved within integrated management alternatives such as the use of wild tomato genotypes. In such a way that they can be incorporated as a sustainable option in commercial tomato production systems in the region.

Author contribution

Conceptualization: N.C-A., C.N.M-E. Formal analysis: M.A.J-A., B.E.P-H., C.G-C., L.D.C-A. Investigation: M.A.J-A., B.E.P-H., C.G-C., L.D.C-A., N.C-A., C.N.M-E. Resources: N.C-A., C.N.M-E. Writing-original draft: M.A.J-A., B.E.P-H., C.G-C., L.D.C-A. Writing-review & editing: N.C-A., C.N.M-E. Supervision: N.C-A., C.N.M-E. All co-authors reviewed the final version and approved the manuscript before submission.

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