

RESEARCH ARTICLE

Effects of *Meloidogyne incognita* on agronomic parameters and structural changes in eggplant (*Solanum gilo* Raddi) roots treated with *Purpureocillium lilacinum*

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Keywords

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ABSTRACT

- *Meloidogyne* spp. induce structural changes during the development of root-knot galls, leading to reduced productivity in various crop species, including eggplants (*Solanum gilo*). In this study, we evaluated the impact of *Meloidogyne incognita* on the development and structure of root-knot galls in eggplants and assessed the effect of the fungus *Purpureocillium lilacinum* as nematocide.
- Three treatments were applied: plants inoculated with *M. incognita*, plants inoculated with *M. incognita* + *P. lilacinum*, and a control. Agronomic impact was assessed 72 days after inoculation (DAI). Histological, morphological, and immunocytochemical analyses were performed at multiple time points after the start of the experiment.
- There was a reduction in weight as well as in root and shoot growth in the plants inoculated with *M. incognita*, whereas the plants from the other two treatments presented similar values. Galls and giant cells were observed at 18 DAI. *Purpureocillium lilacinum* did not interfere with giant cell formation but reduced the number of galls, nematodes, and eggs in the roots. The detection of methyl-esterified homogalacturonans indicated flexible cell walls in young galls, with increased rigidity due to low methyl esterification in mature galls.
- The growth of the aerial part and mass gain of the eggplants were negatively impacted by *M. incognita*; however, the use of the *P. lilacinum* strains reduced crop damage, indicating its efficiency as a biological control agent.

INTRODUCTION

Root-knot nematodes (RKNs) are endoparasites that form feeding sites in the vascular systems of their host plants (Escobar *et al.* 2015; Vilela *et al.* 2021), where they complete their life cycles (Ye *et al.* 2019). The stimuli generated by nematodes lead to the formation of giant cells, which serve as feeding sites, and an increase in cell division and growth in the root cortex, resulting in the formation of galls (Vilela *et al.* 2019, 2023). The life cycle of nematodes, especially *Meloidogyne* spp., is of interest because of their impacts on several crops worldwide (Koenning *et al.* 2004; Dijan-Caporalino Dijan-Caporalino 2012; Onkendi *et al.* 2014). In general, second-stage juveniles (J2s) penetrate the roots in the meristematic zone, migrate to the cells of the elongation zone, and then move towards the vascular cylinder (Bartlem *et al.* 2013). In the vascular system, J2s induce the formation of giant cells (Vilela *et al.* 2021, 2023), which are essential for the nutrition and development of RKNs. The nematodes develop into three to four juvenile and adult stages after inducing the feeding sites (Mitkowski & Abawi

2003). Adult females lay eggs in a gelatinous matrix (Orion *et al.* 2001), whereas adult males leave the roots without causing damage to the host plants (Escobar *et al.* 2015).

The development and establishment of giant cells depend on a series of changes in the structure and composition of the cell walls towards increasing elasticity and porosity, which are essential for the growth and flow of nutrients from cell-to-cell (Caillaud *et al.* 2008; Bozbuga *et al.* 2018; Vilela *et al.* 2019). Cell walls are composed mainly of cellulose, hemicelluloses, and pectins (Defilippi *et al.* 2018), whose proportion and degree of esterification are related to their functional properties (Knox 1997). These three polymers perform several functions, such as determining and maintaining cell shape (Szymanski 2009; Singh & Montgomery 2011), controlling plant cell growth (Wolf & Greiner 2012), resisting internal turgor pressure (Haswell *et al.* 2008), and affecting perception and signalling during plant development, which may activate plant defence mechanisms (Nühse 2012). The cascade of events related to plant–nematode interactions may be evaluated at the cell wall level via monoclonal antibodies. Therefore, knowledge

of the cell wall dynamics in plants attacked by RKNs helps us understand the complex mechanisms of pathogen resistance, as well as comprehend the nematode infection process in roots and the development of feeding sites.

Nematodes are controlled by chemical products, whose use has been restricted by their high cost and toxicity. The long-term use of nematicides poses risks, including potential water contamination and biodiversity loss, which must be carefully considered (Dong & Zhang 2006; Araújo *et al.* 2012). Alternatively, nematophagous fungi can be utilized because they parasitize different life stages of plant-parasitic nematodes (Al-Ani *et al.* 2022), such as *Purpureocillium lilacinum* Thom. (Ophiocordycipitaceae), a soil fungus that effectively controls RKNs by directly affecting their reproductive capacity (Campos *et al.* 1990; Campos 1997; Luangsa-Ard *et al.* 2011; Al-Ani *et al.* 2022). For example, in tomato crops, a single pre-plant application of *P. lilacinus* strain was sufficient to control *Meloidogyne incognita*, indicating that this fungus can control RKNs (Kiewnick & Sikora 2006). From such a perspective, more studies using this biological control agent in RKNs management associated with different plant species may point out other efficient uses.

The eggplant *Solanum gilo* Raddi (Solanaceae) is one of the more rustic species of Solanaceae but is susceptible to attack by *Meloidogyne* (Pinheiro *et al.* 2021), whose resistance can change with biological control. The eggplant *S. gilo* is an important vegetable species for family farms because its bitter fruits have high nutritional and therapeutic value (Odetola *et al.* 2004); however, the high infestation rates of *Meloidogyne* species can hinder its development and reduce crop productivity (Pinheiro *et al.* 2021). Therefore, the current study aimed to test the efficiency of a biological product derived from the fungus *P. lilacinum* in controlling gall formation and the main steps of gall development and giant cell formation. To do that, we conducted a comparative evaluation of inoculated and non-inoculated plants (control), focusing on (i) the effects of *M. incognita* parasitism on the agronomic characteristics of *S. gilo* treated with *P. lilacinum*; (ii) the structural changes in roots during gall development and giant cell formation; and (iii) alterations in cell wall pectin and hemicellulose compounds.

MATERIALS AND METHODS

Experimental design and treatments

The experiment was conducted in a greenhouse at Centro Universitário de Goiátuba—Unicerrado, Goiátuba municipality (18° 00' 45" S, 49° 21' 17" W), Goiás State, Brazil. Seeds of the cultivar Comprido Verde Claro (Topseed®), purchased from Agristar do Brasil LTDA, which is susceptible to the nematode *M. incognita*, were used. Seeds were sown in two plastic trays of 100 wells each, with each well containing the substrate for Bioplant® seedlings. After 20 days of emergence, the plants were transplanted to 2.8 l pots containing sterilized substrate in a 1:1 proportion of sand and dystrophic oxisol (Oxisol) (Santos *et al.* 2018).

Six days after planting, nematodes were inoculated into 100 eggplants in two holes near the roots via a pipette containing a suspension of 5,000 *M. incognita* juveniles (J2s) per 2 ml of inoculum. The nematodes were previously propagated in susceptible cultivars and extracted according to the methods of

Coolen & D'Herde (1972). A microbiological nematicide, purchased from Ballagro Agro Tecnologia LTDA, based on the fungus *Purpureocillium lilacinum* was added to 50 of these plants at a concentration of 7.5×10^9 CFU/g⁻¹, which is indicated for controlling RKNs (*M. javanica* and *M. incognita*). The product was applied shortly before transplanting the seedlings, at the amount recommended by the manufacturer, which we adapted to the pots at 100 ml per pot. The remaining 50 plants were maintained as a control group without inoculation. Thus, the experiment consisted of three treatments: (i) a control without nematodes or fungus, (ii) inoculation with nematodes, and (iii) inoculation with nematodes + application of nematicide based on *P. lilacinum*.

Evaluation of agronomic parameters

Agronomic trait evaluation and gall quantification were performed 72 days after inoculation (DAI) and compared with those of the control group shortly before the traditional eggplant harvest time, which occurred between 80 and 100 days after sowing (Pinheiro *et al.* 2021). During this experiment, the cultivar did not bear fruit. Initially, the plants were removed from the pots, and the roots were washed to remove soil particles and placed on paper towels to remove excess moisture. The following parameters were analysed: (i) fresh root eight of the aerial part (FWAP) and (ii) fresh weight of the roots (FWR), both of which were determined via a Balmak ELP-10; (iii) root length and (iv) aerial part shoot length, which was measured as the distance between the plant collar and the tip of the terminal bud of the main branch; and (v) dry mass of the aerial part (DMAP), which was obtained after the fresh samples were placed in individual Kraft paper bags and dried in an oven at 60°C for 72 h.

Quantification of nematodes and root-knot galls

The number of eggs and nematodes was evaluated in 5 g of roots from each treatment. The roots were cut and crushed in a blender with water and hypochlorite. The solution was passed through a 500 mesh sieve for the extraction of nematodes and root-knot eggs. Kaolin (ceramic powder) was added to the solution, which was then centrifuged, followed by the addition of sucrose. The mixture was centrifuged and sieved again, and the supernatants of nematodes were collected to visualize the nematodes and eggs. A sample was placed on a glass slide and observed under a binocular microscope (Bioval® L-1000b-AC, China), and this procedure was standardized for all the roots with the highest weight.

To evaluate the level of resistance of the eggplant to *M. incognita*, the galls were isolated and counted according to the gall index (GI) proposed by Taylor & Sasser (1978), where the level of infestation was characterized as follows: 0 = 0 galls; 1 = 1–2 galls; 2 = 3–10 galls; 3 = 11–30 galls; 4 = 31–100 galls; and 5 = > 100 galls. Plants with 0–10 galls (0, 1, 2) were considered resistant, whereas plants with >11 galls were considered susceptible (3, 4, 5).

Morphological and anatomical analyses

Roots from control treatment were collected from the root hair zone at 4 and 66 days after seedling transplantation for

Table 1. Monoclonal antibodies and their epitopes used for labelling hemicellulose and pectin in cell walls of roots and root-knot galls of eggplants.

monoclonal antibody	epitopes	references
Pectic polysaccharides/homogalacturonan (HG)		
JIM 5	Partially methyl-esterified up to 40%	VandenBosch <i>et al.</i> (1989); Knox <i>et al.</i> (1990); Willats <i>et al.</i> (2000); Clausen <i>et al.</i> (2003)
JIM 7	15%–80% methyl-esterified	Knox <i>et al.</i> (1990); Willats <i>et al.</i> (2000); Clausen <i>et al.</i> (2003)
Pectic polysaccharides/rhamnogalacturonan-I		
LM 5	(1 → 4) β-D-galactan	Jones <i>et al.</i> (1997); Orfila & Knox (2000); Andersen <i>et al.</i> (2016)
LM 6	(1 → 5) α-L-arabinan	Orfila & Knox (2000); Cornuault <i>et al.</i> (2017)
Non-cellulosic and non-pectic polysaccharides		
LM 11	(1 → 4)-β-D- xylans/arabinoxylans	McCartney <i>et al.</i> (2005)
LM 21	Heteromannans	Marcus <i>et al.</i> (2010)
LM 15	Xyloglucans	Marcus <i>et al.</i> (2008)

anatomical analyses. For galls, the samples from the two treatments, that is, inoculation with *M. incognita* and inoculation with *M. incognita* + *P. lilacinum*, were collected from the median regions of the roots at 04, 11, 18, 25, 32, 39, 46, 54, 66, and 72 DAI for both morphological and anatomical analyses. All the morphological samples were photographed with a stereoscopic microscope (Leica® EZ4 HD, Germany).

For histological analyses, fragments of roots and galls ($n = 5$) were fixed in FAA (formaldehyde, acetic acid, and 70% ethanol, 1:1:18, v/v) for 48 h and stored in 70% ethanol. The samples were subsequently dehydrated in an ethanol series, embedded in 2-hydroxyethyl methacrylate (Historesin, Leica® Instruments, Germany), and transversally sectioned with a rotary microtome (YD-315 model, China) at a thickness of 6 µm. The sections were stained with 1% toluidine blue, pH 4.0 (O'Brien & McCully 1987), and mounted with Entellan®. The samples were photographed with a Leica® DM500 photo-microscope coupled with a Leica® ICC50HD camera.

Cell wall dynamics assessed by immunocytochemical analyses

Samples of roots from the control treatment were collected at 72 days after seedling transplantation in the control group, and samples of galls inoculated with only *M. incognita* were collected at 25 and 72 DAI. The 25 DAI time point was selected for the presence of giant cells in the initial formation stage, whereas the 72 DAI time point was selected as the developmental stage in which the galls and giant cells reached their maximum size in the experiment.

The immunocytochemical detection of hemicelluloses was performed on slides prepared for anatomical analysis and pre-treated with 10 µg/ml⁻¹ pectate lyase (Sigma–Aldrich) in 2 mM CaCl₂ buffer and 50 mM 3-(cyclohexylamino)-1-propanesulfonic acid (CAPS) (Sigma–Aldrich, USA) at pH 10 for 2 h. The next steps were similar for immunocytochemical detection of both pectins and hemicelluloses. The sections were immersed in a blocking solution of Molico® milk powder/phosphate-buffered saline (PBS) for 30 min, followed by incubation with the primary monoclonal antibodies JIM5, JIM7, LM5, LM6, LM11, LM15, and LM21 (Centre for Plant Sciences, University of Leeds, UK) (Table 1), which were diluted 1:10 in milk/PBS, for 2 h. After this incubation, the sections were washed in PBS one more time and incubated with a FITC secondary antibody (1:100 in 3% milk/PBS) for 2 h in the absence of light. A control was obtained by suppressing the primary antibodies during incubation. The slides were

mounted in 50% glycerin and analysed under a fluorescence microscope (DM4000B LED, Leica, Germany) coupled with an HD monochromatic camera (DFC3000G).

Statistical analyses

To assess the development of the eggplant cultures inoculated with *Meloidogyne incognita* and *Meloidogyne incognita* + *P. lilacinum*, in comparison with the control plants, a factorial analysis of variance (ANOVA) was performed, with each agronomic variable used as the response variable and the treatments as predictors. To identify changes in the relationships among the growing parts of the plant, multiple regression analyses were subsequently conducted with the agronomic variable as the response and the treatments interacting with other agronomic variables as predictors. Additionally, multiple regression analyses were used to examine the relationships between the number of nematodes in the soil and roots with the total number of galls. All analyses were performed using the R statistical software, with the 'lme4' package for multiple regressions, the 'MuMIn' package for model selection, and the 'ggplot2' package for graphical representation (R Core Team 2020).

Data on the number of galls, nematodes, and eggs per 5 g of root were statistically analysed using Microsoft Corporation® software to generate descriptive graphs. Subsequently, variance analyses and mean comparisons were performed using Tukey's test at a 5% significance level, employing the freely available SISVAR® software.

RESULTS

Agronomic and developmental characteristics of eggplant

A comparison of the treatments for each variable revealed a reduction in agronomic parameters in plants inoculated only with *M. incognita*, except for root length, which was similar for all the treatments. The FWR of the control plants was similar to that of the plants inoculated with *M. incognita* + *P. lilacinum* ($P = 0.78$), but lower than that of the plants inoculated with only *M. incognita* ($F_{2,87} = 22.28$; $P < 0.001$; Fig. 1A). There was a decrease in the fresh weight of the aerial part (FWAP) in the treatment with only *M. incognita* ($F_{2,87} = 20.42$; $P < 0.001$), with similar FWAP values for the control and *M. incognita* + *P. lilacinum* groups ($P = 0.28$; Fig. 1B). Root length was the only variable that did not differ among treatments ($P = 0.07$; Fig. 1C). However, there was a

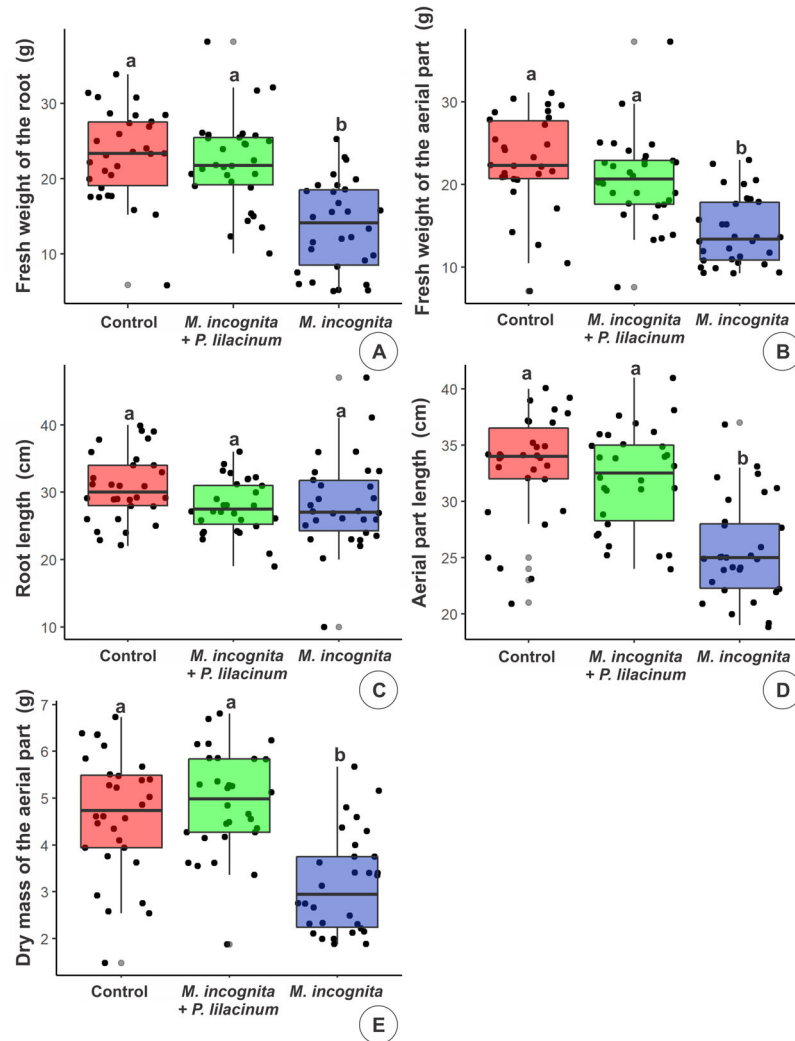


Fig. 1. Multiple regression comparing agronomic features by treatment. (A) Fresh weight of the root (FWR); (B) Fresh weight of the aerial part (FWAP); (C) Root length; (D) Aerial part length; (E) Dry mass of the aerial part (DMAP). Red: Control (non-inoculated plants); Green: Plants inoculated with the nematode (*Meloidogyne incognita*) plus fungus (*Purpureocillium lilacinum*); Blue: Plants inoculated only with the nematode (*M. incognita*). Distinct lowercase letters indicate differences from each other by Tukey's test at 5% significance.

significant difference in the height of the aerial part (Fig. 1D; $F_{2,87} = 21.93$; $P < 0.001$), with the control being similar to the treatment with *M. incognita* + *P. lilacinum* ($P = 0.67$). There was a significant difference in DMAP between the groups (Fig. 1E; $F_{2,87} = 19.85$; $P 0.001$), following the previous pattern, with similar DMAP values between the control group and the *M. incognita* + *P. lilacinum* treatment group ($P = 0.69$).

In the *M. incognita* and *M. incognita* + *P. lilacinum* treatments, there was no increase in the FWR with the increasing number of nematodes in 5 g of roots (Fig. 2A; $b = 0.47$). There was a positive relationship between FWAP and FWR ($b = 4.4$), indicating that larger plants also had larger roots (Fig. 2B). A positive relationship was also observed between the number of galls and the FWR in the treatment with only nematodes ($b = 3.41$; Fig. 2C). This result shows that increasing the number of galls increased the FWR in the treatment with only nematodes; however, there was an inverse pattern for the treatment with *M. incognita* + *P. lilacinum*, in which lighter roots had more galls and heavier roots had fewer galls.

The roots inoculated with *M. incognita* + *P. lilacinum* contained fewer galls than did roots inoculated with only *M. incognita* (Table 2); however, these results still indicated the high susceptibility of the cultivar according to the Taylor and Sasser scale, which was categorized as degree 5 (more than 100 galls). A total of 332 galls were observed in the roots of plants inoculated only with *M. incognita* (variance 74 ± 578), and a total of 274 galls were observed in the roots of plants inoculated with *M. incognita* + *P. lilacinum* (variance 42 ± 490). A reduction in the number of galls, nematodes, and eggs was observed in the roots of plants inoculated with *M. incognita* + *P. lilacinum* (Table 2).

Root-knot gall structure and morphology

At the end of the experiment, the control eggplants showed herbaceous habit (Fig. 3A), had healthy roots, and lacked galls (Fig. 3B and C). Both roots from plants inoculated with only *M. incognita* (Fig. 3D) and inoculated with *M. incognita* +

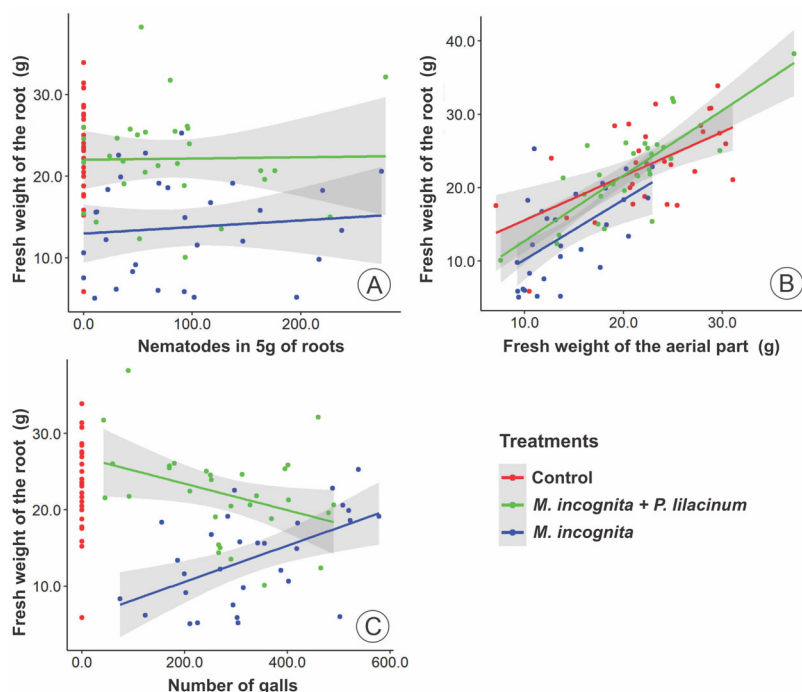


Fig. 2. Multiple regression comparing fresh root weight (g) with agronomic features within treatments. (A) Fresh weight of the root (FWR) × nematodes in 5 g of root; (B) Fresh weight of the root (FWR) × Fresh weight of the aerial part (FWAP); (C) Fresh weight of the root (FWR) × number of galls. Red: Control (non-inoculated plants); Green: Plants inoculated with the nematode (*Meloidogyne incognita*) plus fungus (*Purpureocillium lilacinum*); Blue: Plants inoculated only with the nematode (*M. incognita*). Distinct lowercase letters indicate differences from each other by Tukey's test at 5% significance.

Table 2. Quantity of galls, eggs in 5 g of root, nematodes in 5 g of root and soil in the eggplants inoculated only with *Meloidogyne incognita* and inoculated with *M. incognita* + *Purpureocillium lilacinum*.

treatments	galls	eggs/ 5 g root	nematodes/ 5 g root
<i>Meloidogyne incognita</i>	332.5 ^a	640.6 ^a	91.0 ^a
<i>Meloidogyne incognita</i> + <i>P. lilacinum</i>	274.7 ^b	456.3 ^b	79.4 ^b
CV (%)	53.28	148.5	104.12

Distinct lowercase letters in the column differ from each other by Tukey's test at 5% significance.

CV = coefficient of variation.

P. lilacinum (Fig. 3N) presented galls. In these treatments, the roots did not thicken laterally, indicating no formation of galls at 4 DAI (Fig. 3E and O) or 11 DAI. However, slight lateral thickening was observed in the roots at 18 DAI (Fig. 3F and P), and the thickening was more conspicuous and spherical at 25 DAI (Fig. 3G and Q). At 32 DAI, the galls presented an external egg mass (Fig. 3H) and were dilated (Fig. 3H and R). From 39, 46, and 54 DAI (Fig. 3I, J, K, S, T, and U), the galls increased and exhibited an irregular shape. The galls were large, spherical, and abundant at 66 DAI (Fig. 3L and V). At 72 DAI (Fig. 3M and X), there was an increase in quantity, and some galls coalesced, resulting in root swelling.

In the control treatment, after 4 days of seedling transplantation, the roots of the eggplants exhibited primary growth with an uniseriate epidermis, a triarch vascular cylinder, and a cortex with 3–4 layers of parenchyma cells (Fig. 4A). However,

at 66 days, the roots exhibited typical secondary growth, with the epidermis replaced by a periderm and a secondary xylem developing (Fig. 4B). At 4 DAI, the roots presented an anatomical structure similar to non-inoculated roots, once the galls were not yet evident. At 18 DAI, 4 giant cells in the roots inoculated with only *M. incognita* (Fig. 4C) or with *M. incognita* + *P. lilacinum* (Fig. 4D) were observed, indicating the formation of a feeding site. At 25 DAI, the feeding site was already well established in both treatment groups, with ~ 5 giant cells (Fig. 4E and F). The multinucleated giant cells had dense cytoplasm and thick cell walls (Fig. 4F). These cells are formed in the vascular cylinder, which promotes disorganization of the vascular system (Fig. 4F).

At 39 DAI, the female nematode was globose and close to the giant cells, and an egg mass was observed (Fig. 4G). In the roots inoculated with *M. incognita* + *P. lilacinum*, there were more giant cells (Fig. 4H). The egg mass (Fig. 4I) and female nematodes (Fig. 4J) were also visible at 46 DAI. At 66 DAI, the periderm replaced the epidermis as the dermal system, and there was a large proliferation of cortical parenchyma cells (Fig. 4K). Feeding sites were also observed at 72 DAI in roots inoculated in both treatments (Fig. 4L and M), with several nuclei and fragmented vacuoles in the giant cells, especially in the galls of the plants inoculated with *M. incognita* + *P. lilacinum* (Fig. 4M).

Cell wall dynamics of roots and root-knot galls

Immunocytochemical analysis revealed differences in the labelling and distribution of pectin and hemicellulose epitopes in the roots of control and treatment plants inoculated

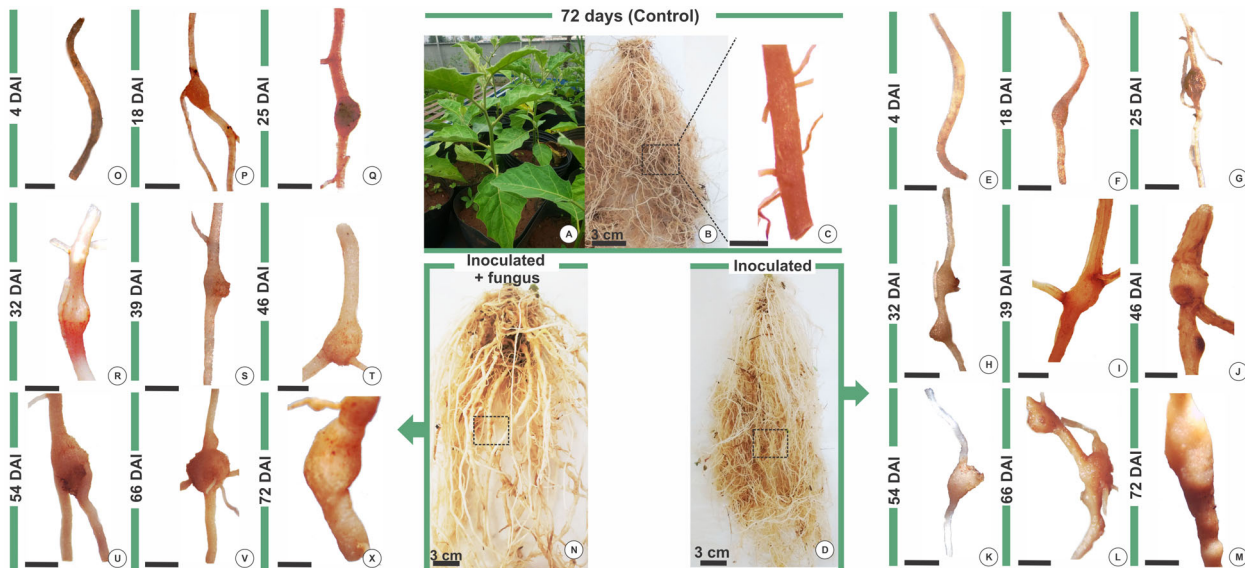


Fig. 3. Morphological features of non-inoculated eggplant (A–C) and its galls inoculated with only *Meloidogyne incognita* (D–M) or with *M. incognita* or *Purpureocillium lilacinum* (N–X). (A) Eggplants in pots; (B) Root system; (C) Root at 72 after seedling transplantation (control group) without gall formation; (D, N) Root system with galls; (E, O) Root at 4 DAI, without lateral thickening; (F, P) Root at 18 DAI with beginning of gall emergence; (G, Q) Presence of small spherical galls at 25 DAI; (H) Eggs occurring outer to the root; (H, R) Larger and spherical galls at 32 DAI; (I, S) Galls at 39 DAI, with irregular shape and larger size; (S) Presence of egg mass externally to the root; (J, T) Larger galls at 46 DAI; (K, U) Maintenance of egg mass at 54 DAI; (L, V) Larger and spherical galls in greater quantity at 66 DAI; (M, X) Increase in the number of galls at 72 DAI, with union of some and swelling of the root.

with *M. incognita*, depending on the tissue and gall developmental stage (Table 3). Epitopes of HGs with high methyl-esterified groups, labelled with the JIM7 antibody, were observed in the cell walls of cortical parenchyma and phloem in control roots, at 72 after seedling transplantation (Fig. 5A; Table 3). At 25 and 72 DAI, these HGs were labelled in the cell walls of cortical parenchyma, xylem parenchyma cells, phloem, and giant cells (Fig. 5B and C; Table 3). Epitopes with low methyl-esterified HGs were labelled by JIM5 in the parenchyma cell walls of the cortex and phloem cells in control roots, at 72 after seedling transplantation in a control group (Fig. 5D; Table 3) and labelled only in the xylem parenchyma cells at 25 DAI (Fig. 5E; Table 2). At 72 DAI, these epitopes were labelled in the cell walls of the cortex, xylem parenchyma cells, phloem, and giant cells (Fig. 5F; Table 3). The epitopes of (1 → 4) β -D-galactans were labelled by LM5 in control roots, at 72 after seedling transplantation in a control group, in the cortex and phloem (Fig. 5G; Table 2). At 25 and 72 DAI, these epitopes were labelled in the cell walls of cortical parenchyma, xylem parenchyma cells, phloem, and giant cells (Fig. 5H and I; Table 3).

Epitopes of (1 → 5) α -L-arabinans were labelled by LM6 in the cortex and phloem in control roots, at 72 h after seedling transplantation (Fig. 6A; Table 3). At 25 DAI, these epitopes were labelled in cell walls of the cortical parenchyma, xylem parenchyma cells, phloem, and giant cells (Fig. 6B; Table 3); however, there was no labelling at 72 DAI. The (1 → 4)- β -D-xylan/arabinoxylan epitopes were not labelled by LM11 in any of the phases (Table 2). The heteromannan epitopes were labelled by LM21 only in the periderm in control roots, at 72 h after seedling transplantation (Table 2). Xyloglucan epitopes were labelled by LM15 only in the cortex, xylem parenchyma cells, phloem, and giant cells at 25 DAI (Fig. 6C; Table 3).

DISCUSSION

The degree of RKN infestation, the number of galls, and susceptibility generally depend on the host plant traits (Mitkowski & Abawi 2003), and *Meloidogyne incognita* galls induced on the roots of eggplants affect shoot growth and weight gain, which may impact crop yield. The microbiological nematocide based on the fungus *P. lilacinum* reduced the impact of *M. incognita* on eggplants, preventing decreases in plant height and weight since it acts mainly by reducing the number of galls, eggs, and nematodes in the roots. Our data demonstrated that the use of *P. lilacinum* was beneficial for reducing the impact of *M. incognita* infection on the eggplant cultivar tested herein. The developmental data revealed that galls and giant cells appear at ~ 18 DAI and cause disorganization of the root vascular cylinder. Tissue hyperplasia and cell hypertrophy in galls occur due to changes in the structure and composition of cell wall pectins and hemicelluloses (Martini *et al.* 2020; Silva *et al.* 2021; Vilela *et al.* 2021; Santos *et al.* 2024), especially with respect to the type of side chain of rhamnogalacturonans and the degree of methyl esterification of homogalacturonans for eggplant root galls.

Effects of RKNs on eggplant's agronomical traits

In the present study, we noted that eggplant was highly susceptible to RKNs, with a grade of 5 (more than 100 galls) on the scale of Taylor & Sasser (1978), which is the most commonly used root-knot index (Abe *et al.* 2019). This degree of susceptibility is also exhibited by other vegetables, such as okra infested by *M. incognita* (Vilela *et al.* 2021), cucumber, and chickpea plants parasitized by *Meloidogyne* spp. (Santos *et al.* 2021; Ramatsitsi & Ramachela 2023) and peas infested with

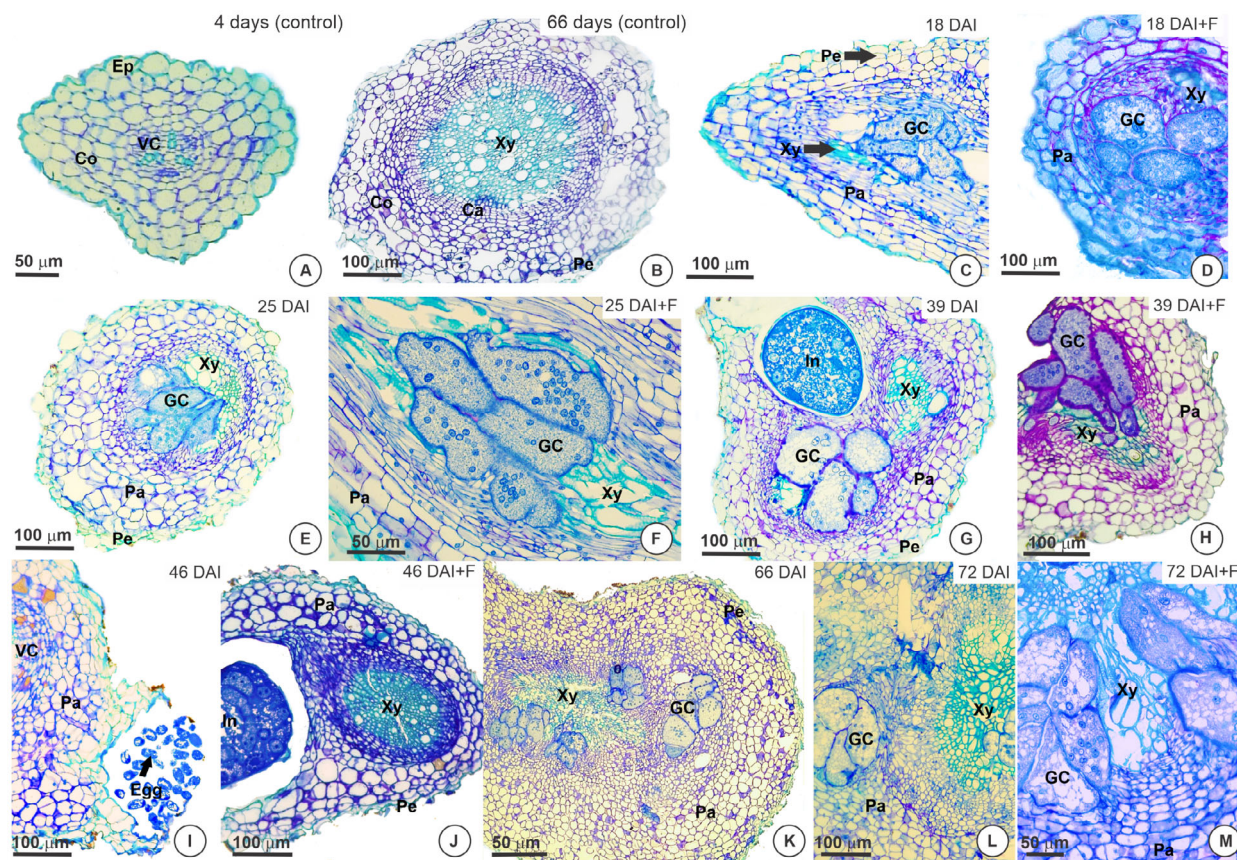


Fig. 4. Anatomical features of non-inoculated roots of eggplant (A, B) and galls after inoculation with *Meloidogyne incognita* alone (C, E, G, I, K, L) and with *M. incognita* + *Purpureocillium lilacinum* (D, F, H, J, M). (A) Non-inoculated root at 4 after seedling transplantation (control group); (B) Uninoculated root at 66 after seedling transplantation (control group); (C) Inoculated root at 18 DAI, with giant cells forming; (D) Root at 18 DAI, with well-formed giant cells; (E) At 25 DAI, the feeding site is well established and with alterations in the vascular system; (F) At 25 DAI, well-formed giant cells, with evident nuclei, granular cytoplasm and a thick cell wall; (G) At 39 DAI, with *M. incognita* female near the giant cells; (H) At 39 DAI, there are several giant cells and a disorganized vascular system; (I) At 46 DAI, there is a clear presence of the egg mass in a gelatinous mass; (J) At 46 DAI, the presence of the inducer can be seen in the female chamber; (K) At 66 DAI, there are several feeding sites and a large number of parenchyma cells; (L, M) At 72 DAI, with details of the giant cells and their fragmented vacuoles. Ca, cambium; Co, cortex; Egg, egg mass of the female nematode; Ep, epidermis; GC, giant cell; In, inducer; Pa, parenchyma; Pe, periderm; VC, vascular cylinder; Xy, xylem.

Table 3. Immunocytochemistry results for the roots of control eggplants at 72 days of seedling transplantation, and those with galls after inoculation (25 DAI and 72 DAI) with only *Meloidogyne incognita*.

monoclonal antibodies	72 days (control)					25 DAI						72 DAI					
	Pe	Co	Xy		Ph	Pe	Co	Xy		Ph	GC	Pe	Co	Xy		Ph	GC
			PC	Ve				PC	Ve					PC	Ve		
JIM7	—	+	—	—	+	—	+	+	—	+	+	—	+	+	—	+	+
JIM5	—	+	—	—	+	—	—	+	—	—	—	—	+	+	—	+	+
LM5	—	+	—	—	+	—	+	+	—	+	+	—	+	+	—	+	+
LM6	—	+	—	—	+	—	+	+	—	+	+	—	—	—	—	—	—
LM11	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
LM21	—	—	—	—	—	+	—	—	—	—	—	—	—	—	—	—	—

Immunocytochemical result: (–) negative; (+) positive.

Co = cortex; GC = giant cell; PC = Parenchymatic cell; Pe = periderm; Ph = phloem; Ve = vessel; Xy = xylem.

M. javanica (Sharma *et al.* 2000). *Meloidogyne incognita* infection on eggplants decreases several agronomical parameters once the galls act as sinks of photoassimilates (Oliveira

et al. 2016). Gall formation on roots can also cause a reduction in nutrient uptake and water translocation, negatively affecting the growth of the aerial plant parts (Araújo *et al.* 2012). Low

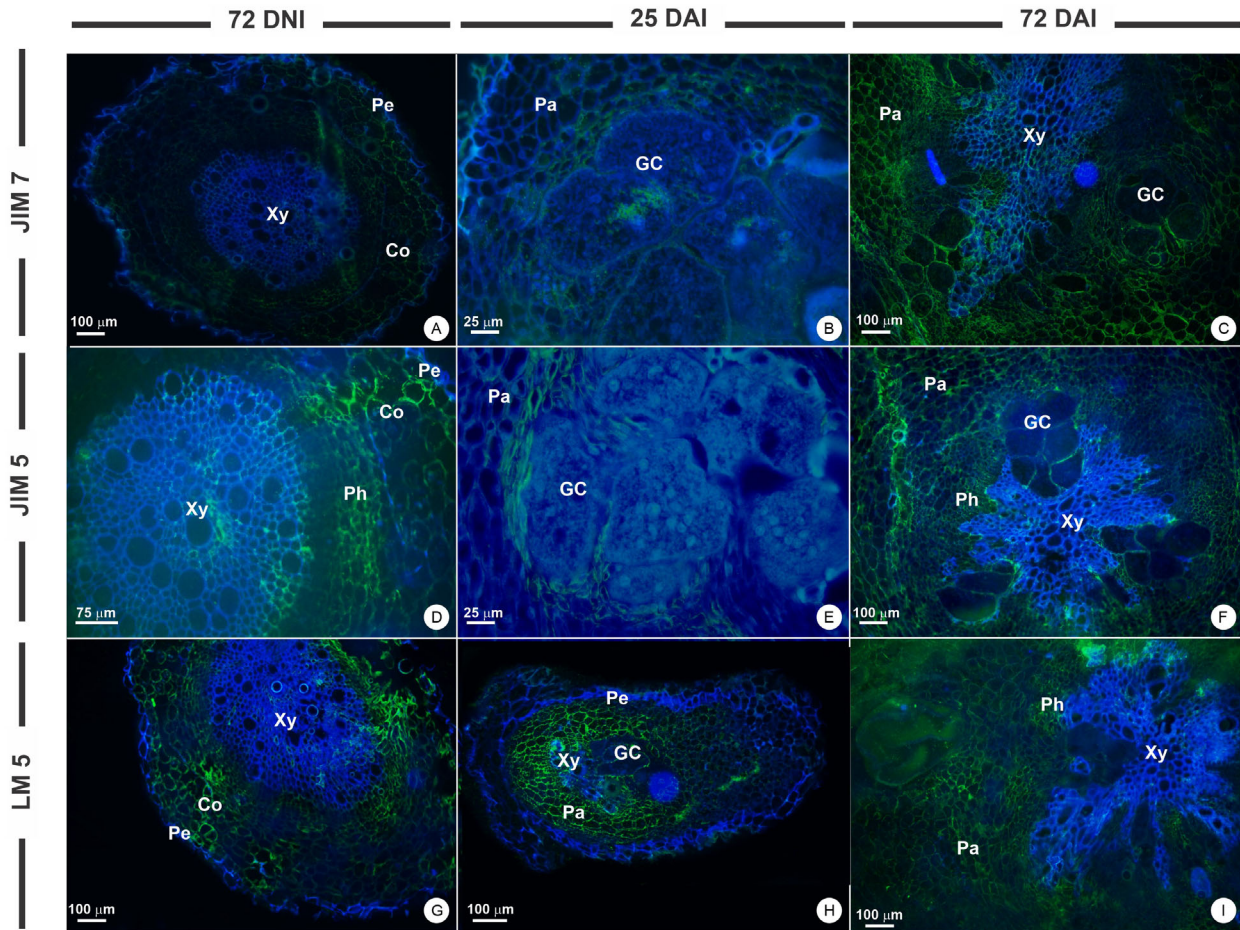


Fig. 5. Distribution of homogalacturonans, labelled JIM7 and JIM5, and rhamnogalacturonans, labelled LM5, in non-inoculated roots of *Meloidogyne incognita* at 72 after seedling transplantation (control group) (A, D, G) and inoculated at 25 DAI (B, E, H) and 72 DAI (C, F, I). Co, cortex; Eg, egg; GC, giant cells; Pa, parenchyma; Pe, periderm; Ph, phloem; Xy, xylem.

vigour and reduced shoot development after nematode infection have been reported in different crops, such as beet, in which there was a significant reduction in shoot and root fresh mass (Korayem 2006) and a decrease in the mass and diameter of tubers (Débia *et al.* 2019). Different crops deal with loss of productivity due to the infection by the root-knot nematode (Mitkowski & Abawi 2003; Ralmi *et al.* 2016), and the fungus-based product as a biological control tested herein can offer an alternative way to control this parasite.

Purpureocillium lilacinum is a soil fungus that effectively controls *Meloidogyne* species in different plant species (Santiago *et al.* 2006; Al-Ani *et al.* 2022), as corroborated in the present study. This fungus uses different mechanisms to destroy RKNs and is efficient as a biological control (Huang *et al.* 2004; Soares *et al.* 2023). *P. lilacinum* can penetrate in nematode eggs and destroy the embryo, exerting strong pressure on the reproductive capacity of females, as it kills after colonization and reduces the number of nematodes and galls (Dunn *et al.* 1982; Al-Ani *et al.* 2022). The agronomic parameters of the fungus-treated plants were similar to the control, suggesting that *P. lilacinum* increases the host plant's resistance to nematode infestation. Our results fit those of Saleh *et al.* (2023), who evaluated five *P. lilacinum* isolates as biocontrol agents against

the root-knot nematode *M. javanica*. This phenomenon has already been reported for other groups of fungi, such as *Trichoderma* spp., which increase the resistance of plants to abiotic stresses, improve nutrient utilization, and reinforce the growth, development, and productivity of some crops (Harman *et al.* 2004; Yao *et al.* 2023). Our results confirm the ability of *P. lilacinum* to prevent *Meloidogyne* infection, reducing the *Meloidogyne* population during infection. So, the use of *P. lilacinum* is an effective measure for controlling the number of galls, allowing the maintenance of nematode density below the level of economic damage, as reported by Rusinque *et al.* (2018).

Root-knot galls morphology

Meloidogyne incognita can trigger several events that can lead to the formation of multiple galls on the roots, represented by large clusters, causing root swells, which occur in eggplants after 66 DAI. Galls induced by *Meloidogyne* have been reported to damage solanaceous crops worldwide (Barbay *et al.* 2015), such as peppers (*Capsicum annuum*) (Carrillo-Fasio *et al.* 2020), tomatoes (*Solanum lycopersicum*) (Rusinque *et al.* 2018), and potatoes (*Solanum tuberosum*) (Schafer *et al.* 2017),

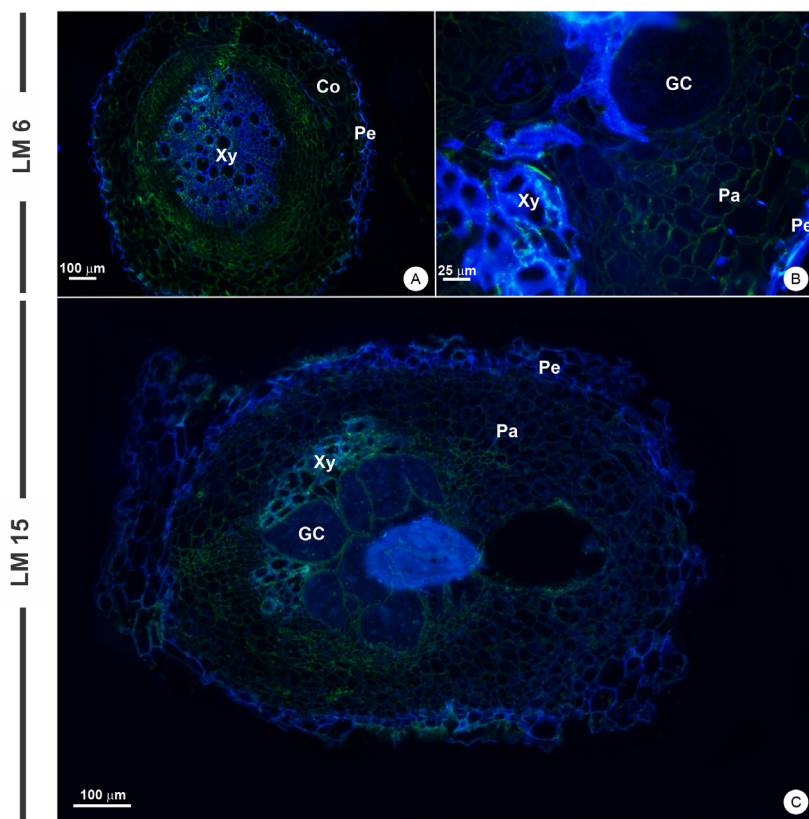


Fig. 6. Distribution of pectic epitopes labelled with LM6 in non-inoculated roots at 72 hours after seedling transplantation (control group) (A) and inoculated with *Meloidogyne incognita* at 25 DAI (B), as well as hemicellulosic epitopes labelled with LM15 at 72 DAI (C). Co, cortex; GC, giant cells; Pa, parenchyma; Pe, periderm; Xy, xylem.

in addition to other plant families, such as Malvaceae (*Abelmoschus esculentus*) (Vilela *et al.* 2021), Fabaceae (*Glycine max*) (Vilela *et al.* 2019), and the chickpea (*Cicer arietinum*) (Vovlas *et al.* 2005), whose morphology is similar to that of the galls induced on eggplants.

Root-knot galls differ in size and shape (Ferraz 2001); however, in the present study, there were no differences in these aspects between the galls that developed in the roots inoculated with *M. incognita* and those inoculated with *M. incognita* + *P. lilacinum*. The differences became more pronounced in both types of roots during development, as the galls were slight protuberances in the initial stage, changed to a spherical shape in the intermediate stage, and coalesced, forming swellings along the roots in more advanced stages. These findings are similar to those reported for okra roots (Vilela *et al.* 2021), in which the appearance of elongated galls and swelling along the root system may be due to multiple infections triggered by numerous juveniles in the roots, generating high rates of infection, and consequently, fusion of the galls (Williamson & Hussey 1996).

Galls may be rare and small even on susceptible plants, such as in maize parasitized by *M. javanica*, which shows slight thickening of the rootlets (Asmus *et al.* 2000). In eggplants and peppers, galls are much smaller than those observed in other vegetables, such as tomatoes (Rusique *et al.* 2018; Carrillo-Fasio *et al.* 2020), which when parasitized by *M. incognita*, exhibit larger and irregularly shaped galls. In

soybean, root galls of varying sizes depend on the nematode population density and cultivar susceptibility (Lin *et al.* 2022), which are important factors influencing the number of galls.

Impact of root-knot gall formation on root anatomy

The root-knot nematode establishes and maintains a close relationship with the host plant during parasitism (Rutter *et al.* 2022), and when successful, the interaction is characterized by the formation of highly specific giant cells induced and maintained by females (Escobar *et al.* 2015; Mukhtar & Kayang 2020; Lin & Siddique 2024). The giant cells develop through the stimulation of secretions from oesophageal glands that contain growth regulators, which induce cell hypertrophy and intense hyperplasia (Mitkowski & Abawi 2003). The formation of giant cells associated with hyperplasia and hypertrophy, mainly of parenchyma cells, is the main event that characterizes gall formation in eggplants.

Giant cells were observed both in roots inoculated with *M. incognita* and in those inoculated with *M. incognita* + *P. lilacinum*, suggesting that the fungus did not interfere with the formation of the feeding sites on the galls. Even though *P. lilacinum* is known for its ability to colonize and kill females (Dunn *et al.* 1982), the feeding sites were still well active at 66 and 72 DAI in the roots. The feeding sites were already present in *Arachis* species induced by *M. arenaria* at 15–19 DAI (Proite *et al.* 2008). In okra, 11 DAI feeding sites with more than four

giant cells in all stages have already been reported (Vilela *et al.* 2021), which occurs in eggplants after 18 DAI. In eggplants, females with egg masses were observed near the feeding site at 39 DAI; these females exhibited a globose shape, as usual (Mitkowski & Abawi 2003). In the potato cultivar Cara infested by *M. javanica*, each adult female was surrounded by three to six large giant cells with thickened cell walls, granular cytoplasm, and numerous nuclei (Vovlas *et al.* 2005); these cellular features are similar to those found in soybean giant cells parasitized by *M. javanica* (Vilela *et al.* 2019), as well as those found in the eggplants at 25 DAI. Giant cells are usually multinucleated, which allows high production of proteins that can be used to feed the nematode (Mitkowski & Abawi 2003). The presence of giant cells seems to disorganize the central vascular cylinder of infected roots and may compromise water transport and nutrient uptake, thereby reducing growth (Anwar & Javed 2010; Premachandra & Gowen 2015). In this study, different parameters showed that gall development compromises host growth, which can be associated with the formation of giant cells and the consequent disorganization of the root histological profile.

Cell wall dynamics in root-knot galls

Monoclonal antibodies have been used to determine the dynamics of cell wall compounds during the development of galls induced by insects (Formiga *et al.* 2013; Oliveira *et al.* 2014; Carneiro & Isaias 2015; Martini *et al.* 2020; Silva *et al.* 2021; Santos *et al.* 2024) and more specifically, in nematode-induced galls (Bozbuga 2017; Meidani *et al.* 2019; Vilela *et al.* 2021). In the present study, there was a large change in the composition of the cell walls in the root tissues, with a predominance of new pectin and hemicellulose profiles during the development of the eggplant galls.

Pectins are polysaccharides that are abundant in galacturonic acid and are distributed into three classes: rhamnogalacturonans I (RG-I), rhamnogalacturonans II (RG-II), and homogalacturonans (HGs) (Ridley *et al.* 2001; Willats *et al.* 2001; Albersheim *et al.* 2011). The HGs are recognized by the monoclonal antibody JIM5, which binds to epitopes present in polymers of relatively unesterified HGs, whereas JIM7 binds to HGs with moderate to high methyl esterification (Knox *et al.* 1990; Willats *et al.* 2000). The degree of esterification is related to the different functionalities of the cell walls, with a gain in flexibility associated with high methyl-esterified HGs and stiffness associated with non-methyl-esterified HGs (Knox *et al.* 1990; Willats *et al.* 2000; Sabba & Lulai 2005). In general, the HGs are biosynthesized in a highly methyl-esterified form, making the cell wall flexible and able to expand (Albersheim *et al.* 2011; Pérez-Pérez *et al.* 2019). During plant development and organ maturation, methyl groups can be removed by the action of pectin methylesterases (PMEs), which remove methyl groups, leading to the formation of unmethyl-esterified residues (Levesque-Tremblay *et al.* 2015). The non-inoculated eggplant roots presented cortices and phloem with different levels of HG methyl esterification, which indicates a balance among cell stiffening, porosity, and flexibility (Albersheim *et al.* 2011).

At 25 DAI, the eggplant young galls presented a predominance of highly methyl-esterified HGs, also labelled in the giant cell walls. At 72 DAI, at the final development of the galls, the

giant cell walls become demethylesterified. This process demonstrates an initial investment in cell extensibility for the formation of feeding sites in eggplant galls, with subsequent final cell stiffening (Knox *et al.* 1990; Sabba & Lulai 2005). More than HGs with low methyl esterification were observed more frequently in the last stage of the inoculated root in eggplants, the nematode-induced giant cells are abundant in galactans and xyloglucans, an immunocytochemical profile that has been previously reported in maize (Bozbuga 2017). Similarly, the demethylesterification of the cell walls of giant cells and the xylem parenchyma cells may increase porosity, and thus, the exchange of nutrients within RKNs, as observed in okra (Vilela *et al.* 2021).

The (1 → 5)- α -L-arabinan epitopes recognized by LM6 are related to cell adhesion and are generally associated with decreased cell expansion capacity (Brummell *et al.* 2004). These epitopes were not detected in the mature root-knot gall of the eggplant at 72 DAI but in distinct tissues in the young galls at 25 DAI, which may indicate an expansive capacity in the cell walls of the old eggplant galls. Moreover, there was an overlap in the labelling of (1 → 5)- α -L-arabinan epitopes and (1 → 4)- β -D-galactans in young gall tissues by LM6 and LM5. The (1 → 4)- β -D-galactans allow the cell walls to expand and elongate (McCartney *et al.* 2003), which is expected for the young tissues of eggplant galls at 25 DAI. The overlap with the LM5 and LM6 labelling may, therefore, indicate an associated function between expansion and adhesion throughout the development of eggplant galls. The xyloglucans are essential in regulating cell wall expansion (Carpita 1996; Cosgrove 1999), aiding cell elongation and growth (Pauly *et al.* 2013). Their detection in the young galls at 25 DAI and absence in the mature stage, both with and without inoculation, reinforces this regulatory role along the formation and development of the cell walls of eggplant galls, including the giant cells.

CONCLUSION

The negative impacts of *M. incognita* parasitism on the agronomic characteristics of eggplants are related to changes in the root's structural profile and the establishment of nematode feeding sites, specifically the giant cells, within the host plant vascular system. The impacts extend to the dynamics of the cell walls in the hypertrophied cells of the gall cortex, particularly in the process of giant cell differentiation, where the detection of high methyl-esterified HGs appears essential for giant cell growth and elongation. Combined with galactans and arabinans, the HGs accumulation in giant cell walls may facilitate the flow of cellular fluids necessary for nematode feeding due to increased cell wall porosity. Finally, the product based on the fungus *P. lilacinum* has proved efficient in the biological control of *M. incognita* gall formation by reducing the number of nematodes and galls and impairing the impact on the host plant root structure.

AUTHOR CONTRIBUTIONS

RMIFV designed the experiments and developed the methodologies. VCK, TAM, and RMSI supported anatomical and immunocytochemical analyses. VCM performed the statistical analysis. AAS helped obtain the agronomic data. DCO

coordinated the project and supervised all the methodological steps. All the authors analysed and discussed the data.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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