



Impact of *Meloidogyne incognita* (nematode) infection on root tissues and cell wall composition of okra (*Abelmoschus esculentus* L. Moench, Malvaceae)

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Abstract

Root-knot nematodes are endoparasites whose mature females lodge and grow inside the root of some cultivated plants, leading to losses in productivity. Herein, we investigated if the infection of okra, *Abelmoschus esculentus* (Malvaceae), promoted by the root-knot nematode *Meloidogyne incognita* (Meloidogynidae) changes some agronomic traits of the host plant, as well as the cell wall composition of the root tissues. The okra Santa Cruz 47® cultivar was infected with a suspension of 5000 *M. incognita* juveniles. The inoculated and non-inoculated okra plants were then submitted to morphological analysis at the end of experiment, as well as histological (at 4, 11, 18, 39, and 66 days after inoculation) and immunocytochemical analysis (control and 66 days after inoculation). Root-knot nematode infection reduced the dry weight of the stem system but, unexpectedly, the number and weight of fruits increased. At 11 days after inoculation, we detected the presence of giant cells that increased in number and size until the end of the experiment, at 66 days after inoculation. These cells came from the xylem parenchyma and showed intense and moderate labeling for epitopes recognized by JIM5 and JIM7. The presence of homogalacturonans (HGs) with different degrees of methyl esterification seems to be related to the injuries caused by the nematode feeding activity and to the processes of giant cell hypertrophy. In addition, the presence of HGs with high methyl-esterified groups can increase the cell wall porosity and facilitate the flux of nutrients for the root-knot nematode.

Keywords Cultivated plants · Giant cells · Pectin · Root-knot nematode

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Introduction

The root-knot nematode is one of the most relevant pathogens of cultivated plants around the world, affecting different cultures and leading to great losses in productivity (Jones et al. 2013; Silva et al. 2016). The genus *Meloidogyne* (Meloidogynidae) stands out as the main disease-causing agent for okra, *Abelmoschus esculentus* L. Moench (Malvaceae) (Pinheiro et al. 2013), leading to a loss of up to 27% in productivity (Sikora and Fernandez 2005). Okra is highly suitable for small farm agriculture (Filgueira 2008) since it involves low production costs, easy growth, and highly profitable revenue (Mota et al. 2000). It is also a versatile and nutrient-rich vegetable of great potential value, which may be used for its oil, fuel and biomass, and as animal feed (Martin 1982). Despite many benefits, okra culture is exceptionally susceptible to attack by nematodes, such as *Meloidogyne incognita* root-knot nematode, that could impact the productivity of the crop.

Root-knot nematodes are endoparasites whose globose and sedentary mature females lodge and grow inside the root of plants (Mitkowski and Abawi 2003). These nematodes feed on the stele cells of roots, inducing galls by cell division and hypertrophy of the root cortex (Williamson and Gleason 2003; Galbieri and Belot 2016). During the feeding activity of the root-knot nematodes, 5 to 8 feeding cells, the giant cells, develop in the vascular cylinder (Escobar et al. 2015). Giant cells are induced by the effector proteins secreted by the nematode, which interact with genes and proteins of the host cells (Bellafiore and Briggs 2010; Gheysen and Mitchum 2011; Ali et al. 2017), change their physiological and structural traits (Siddique and Grundler 2015; Juvale and Baum 2018; Smant et al. 2018), and lead to the success of the parasitism. Giant cell formation depends on the continuous feeding stimulus of the gall-inducing nematode, starting with successive nuclear divisions without cytoplasmic division (Mitkowski and Abawi 2003; Abad et al. 2009; Escobar et al. 2015), which makes the cells multinucleated. During giant cell development, the cell cytoplasm turns dense and the cell wall invaginates, increasing the cell surface and the absorption of photoassimilates, minerals, and other substances (Reddigari et al. 1985; Berg et al. 2009; Vilela et al. 2019). For instance, the giant cells induced by the root-knot nematode on *Glycine max* L. exhibited invaginations on their walls and numerous associated mitochondria (Vilela et al. 2019). The occurrence of numerous mitochondria in giant cells indicates a high energy demand (Golinowski et al. 1996; Hussey and Grundler 1998; Vieira et al. 2013) since giant cells are a specialized sink, supplying resources to the nematode until reproduction.

Few studies using a cellular approach have addressed the changes in cell wall composition promoted by infectious nematodes in host plants. As examples, distinct syncytial cell wall compositions were recorded in infectious processes induced by *Heterodera schachtii* in *Arabidopsis thaliana*, by *Globodera pallida* in potatoes, by *Heterodera glycines* in soybean, and by *Heterodera avenae* and *H. filipjevi* in spring wheat (Davies et al. 2012; Zhang et al. 2016). In the present study, we focused on how the infection by root-knot nematodes changes one of the most important components of the primary cell wall, i.e., pectic polysaccharide (see Albersheim et al. 2011). The main pectic polysaccharides are homogalacturonan (HG), rhamnogalacturonan-I (RG-I), rhamnogalacturonan-II (RG-II), xylogalacturonan (XGA), and apiogalacturonan (AP) (Ridley et al. 2001; Willats et al. 2001; Albersheim et al. 2011). Changes in cross-linking capacity with cations and the degree of methyl-esterification of these polysaccharides, especially HGs, can define the physical and biological properties of the cell walls (Willats et al. 2001; Albersheim et al. 2011; Liu et al. 2013; Oliveira et al. 2014; Martini et al. 2019). The dynamics and impact of *Meloidogyne incognita* on the host cell wall composition of the host have not been addressed yet. Thus, understanding the

cell wall dynamics and composition of giant cells in relation to surrounding cells can provide new insights into the parasite-host interaction and the mechanism of nematode gall formation.

The current study evaluates the tissue and cellular changes promoted by the *Meloidogyne incognita* root-knot nematode on *Abelmoschus esculentus*. Cell wall composition was targeted here, with emphasis on protein and pectin modulation during giant cell development, which would help us understand how *M. incognita* respond to the *A. esculentus* infestation. Our objectives were (i) to assess the influence of *M. incognita* root-knot nematodes on the agricultural traits of the okra plant; (ii) determine the changes in the development, morphology, and histology of roots infected by *M. incognita*; and (iii) evaluate the changes of pectin and protein cell wall composition of root-knot nematode galls, with emphasis on giant cell events.

Material and methods

Experimental setup

The experiment was carried out in the greenhouse of Centro Universitário UniCerrado, Goiátuba municipality (18° 00' 45" S, 49° 21' 17" W), Goiás State, Brazil, between February and May 2019. *Abelmoschus esculentus* (Malvaceae), Okra Santa Cruz 47® cultivar (Fig. 1a), was selected for the experiment since it is the cultivar most extensively used by small cropping farmers in Brazil (Pinheiro 2017) and because of its high susceptibility to infection with the root-knot nematode *Meloidogyne incognita* (Meloidogynidae) (Oliveira et al. 2007).

Okra seeds were sown into 98-cell trays filled with Bioplant® substrate. Two weeks after emergence, seedlings were transplanted to 2.8-L vases with a substrate proportion of 1:1 sand/soil sterilized by autoclaving at 120 °C for 1 hour, along with 12 g of the fertilizer Forth Cote NPK (15-09-12). Six days after transplantation, the root-knot nematodes were inoculated into two holes around the roots by pipetting a 5-ml suspension of 5000 *M. incognita* juveniles (J2). Just the inoculated juvenile, females can induce gall on the roots. A total of 50 okra plants were inoculated and 50 others were kept as controls (i.e., non-inoculated plants) among two treatments with 10 repetitions. Each plot was composed of five vases and each vase contained one plant in a randomized block design.

Structural and agronomical traits of nematode galls

The structural traits resulting from gall infection were analyzed at 4, 11, 18, 39, and 66 days after inoculation (DAI). After evaluating the root changes in each DAI, we selected

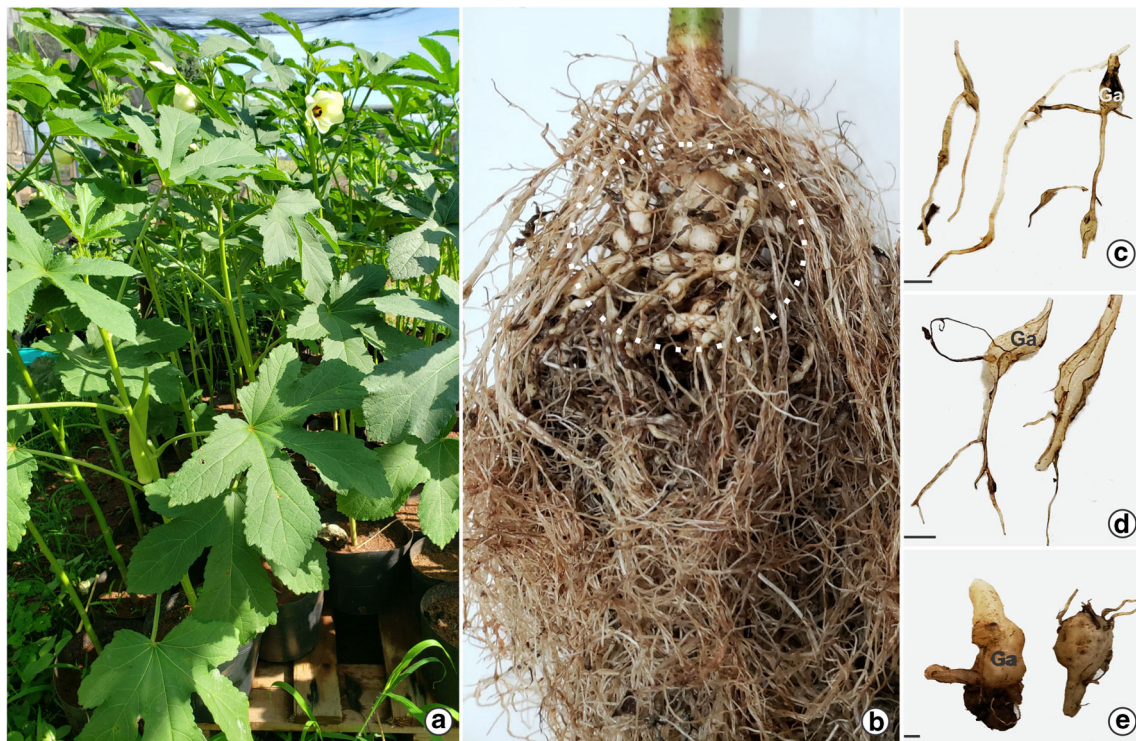


Fig. 1 Morphological characteristics of okra inoculated with the *Meloidogyne incognita* root-knot nematode. **a** Okra cultivar used in the experiments; **b** roots of okra with galls (white dotted circle); **c** small galls; **d** medium galls; **e** large galls. Abbreviations: Ga = gall. Bars = 0.2 cm

inoculated ($n = 40$) and non-inoculated ($n = 40$) okra plants at 66 DAI for specific agronomical trait analyses. At these stages, all plants were carefully removed from the vases and placed on paper towels to dry. The height of the stem system (from the collar to the terminal bud of the main axis) and the length of the roots (Fig. 1b) were measured. The fresh weight of the stem system, fruits, and roots was weighed on a digital scale (Balmak ELP-10), followed by drying in a kiln for 72 hours and weighing the dry weight.

The fruits were manually counted and weighed using a digital scale (Balmak ELP-10) for the assessment of productivity. Galls were isolated and counted for the estimation of gall index (GI) according to Taylor and Sasser (1978), with plants showing resistant reaction status when they have 0 to 10 galls and susceptible reaction status when they have ≥ 11 galls. The method of Coolen and D'Herde (1972) was used to extract and count the nematodes from 20 roots of okra plants. Galls were classified as large when measuring ≥ 1 cm and as small/medium galls when measuring ≤ 1 cm (Fig. 1c–e).

Histological features

Samples of inoculated okra roots (i.e., 4, 11, 18, 39, and 66 days after inoculation) and non-inoculated roots (i.e., 4 and 66 days after inoculation) were fixed in 4% paraformaldehyde (Roland and Vian 1991), dehydrated in a graded ethanol series

(50, 70, 80, 90, and 95%), and embedded in 2-hydroxyethyl methacrylate Histo-resin® (Leica Instruments, Heidelberg, Germany). Next, all samples were cut into 6- μ m transverse and longitudinal sections with a rotary microtome (YD315, ANCAP, Brazil) and stained with 0.05% toluidine blue, pH 4.7 (O'Brien et al. 1964). The sections were mounted with Entellan® and analyzed with a Leica® DM50 light microscope coupled to an HD camera.

Immunocytochemical approach

Roots were selected for immunocytochemistry at 66 days after inoculation and at 66 days after normal non-inoculated growth. The previously described Leica® Histo-resin protocol was used, but without sample staining or mounting. The samples were then incubated in blocking solution (3% powdered milk solution, Molico® in PBS) for 30 minutes to prevent cross-linking, followed by incubation with primary monoclonal antibodies (JIM 5, JIM 7, LM1, LM5, LM6) at 1:5 dilution in blocking solution for 1 hour in the dark. The samples were washed with PBS three times of 5 min each and incubated with the secondary anti-rat IgG FITC antibody (Sigma-Aldrich®) in blocking solution (1:100) for 2 hours in the dark. Samples of inoculated and non-inoculated roots without primary antibodies were used as controls. Finally, the samples were washed in PBS, mounted in glycerin/distilled water (1:1 v/v), and analyzed with a Leica® DM4000 B fluorescence

microscope coupled to a Leica® DFC3000 G camera. We also use a DAPI filter (excitation spectrum 385–400 nm) for auto-fluorescence localization (Chomick et al. 2014; Joca et al. 2019) in an overlap function of the microscope software to better show the results of monoclonal antibodies.

The monoclonal antibodies labeled epitopes for low methyl-esterified HG (JIM 5), high methyl-esterified HG (JIM 7), extensin (LM1), (1→4) β-D-galactan (LM5), and (1→5) α-L-arabinan (LM6). Based on immunocytochemical images, the intensity of the fluorescence reaction was measured in the different tissues using gray scale methodology (Gy = gray value) and ImageJ version 1.51 k software (<http://rsb.info.nih.gov/ij>). Fluorescence intensity was measured in triplicate in each tissue and classified into the following three intensity categories: (–) negative (= 0 Gy values); (+) weak (< 15 Gy values); (++) moderate (15–30 Gy values), and (+++) intense (> 30 Gy values).

Data analyses

Statistical analyses were performed only for the morphological results. First, all data were tested for normal distribution of errors and homogeneity of variances and then analyzed by one-way ANOVA and the Tukey test at the 5% level of significance. Analyses were performed using the SISVAR software (Ferreira 2010).

Results

Agronomical traits

After 66 days, inoculated plants were taller than non-inoculated plants, although there were no differences in root length or fresh weight of the stem system and roots (Table 1). The dry weight of the stem system was greater for the non-inoculated treatment, while inoculated plants exhibited larger and more numerous fruits than non-inoculated ones (Table 1). In addition, 66 days after inoculation, there was counted 425

small/medium galls and 15 large galls for each gall system and 169 nematodes per 5 g of roots.

Gall morphology and development

The regular non-inoculated root of *A. esculentus* was light brown in color with small darkened portions and no pronounced protrusions (Fig. 2a). The roots of inoculated plants did not show any noticeable morphological alteration until day 11, when a slight thickening was first detected (Fig. 2b, c). The root-knot nematode galls greatly increased in size after 18, 39, and 46 days (Fig. 2d, e, f), with new lateral roots developing directly from the galls and the principal roots. After 66 days, we noted an increase in gall number on the root system, with these galls being larger, clustered, and of irregular shape. No changes in morphology were noticed in non-inoculated roots except for the regular thickening caused by normal growth (data not shown).

Histological changes after nematode induction

In general, the gall formation disorganized the histological profile of the roots, creating a new and unique structure. At day 4 of the experiment, the non-inoculated plants of *A. esculentus* showed roots with a uniseriate epidermis, parenchymatic cortex, a vascular cylinder with five protoxylem poles, and no pith (Fig. 3a). On day 66, the vascular cambium had already been established and secondary growth was usual, with the production of more secondary xylem than secondary phloem (Fig. 3b). The main changes seen in inoculated roots were first detected on day 11, with well-formed multinucleated giant cells produced from xylem parenchymal cells, which changed the typical polyarchy organization of the root (Fig. 3c). After giant cell development, the primary phloem was pushed to the periphery of the gall and the cortex grew due to the hypertrophy process (Fig. 3c). In that stage the female of root-knot nematode are in contact with giant cells that showed thick cell walls and dense cytoplasm (Fig. 3c, detail). On day 18, the giant cells remained in the center of the organ, surrounded by primary xylem (Fig. 3d). On day 39,

Table 1 Means of morphological data and standard derivation of agronomical data from 40 non-inoculated okra plants and from 40 okra plants inoculated by *Meloidogyne incognita* root-knot nematodes at 66 days after inoculation

Treatments	Morphological data						
	HSS (cm)	RL (cm)	FWSS (g)	FWR (g)	DWSS (g)	NF	WF (g)
Not inoculated	53 ± 31 ^b	40 ± 8 ^a	53 ± 33 ^a	44 ± 0.18 ^a	32 ± 17 ^a	0.78 ± 1.15 ^b	10 ± 17 ^b
Inoculated	71 ± 25 ^a	37 ± 10 ^a	54 ± 28 ^a	47 ± 0.21 ^a	23 ± 21 ^b	1.22 ± 1.21 ^a	19 ± 24 ^a
CV (%)	14.48	15.36	20.76	14.08	28.57	39.33	42.18

Abbreviations: HSS height of stem system; RL root length; FWSS fresh weight of stem system; FWR fresh weight of root; DWSS Dry weight of stem system; NF number of fruits; WF weight of fruits; CV *coefficient of variation*

*Averages followed by the same lowercase letter in the columns do not differ between treatments

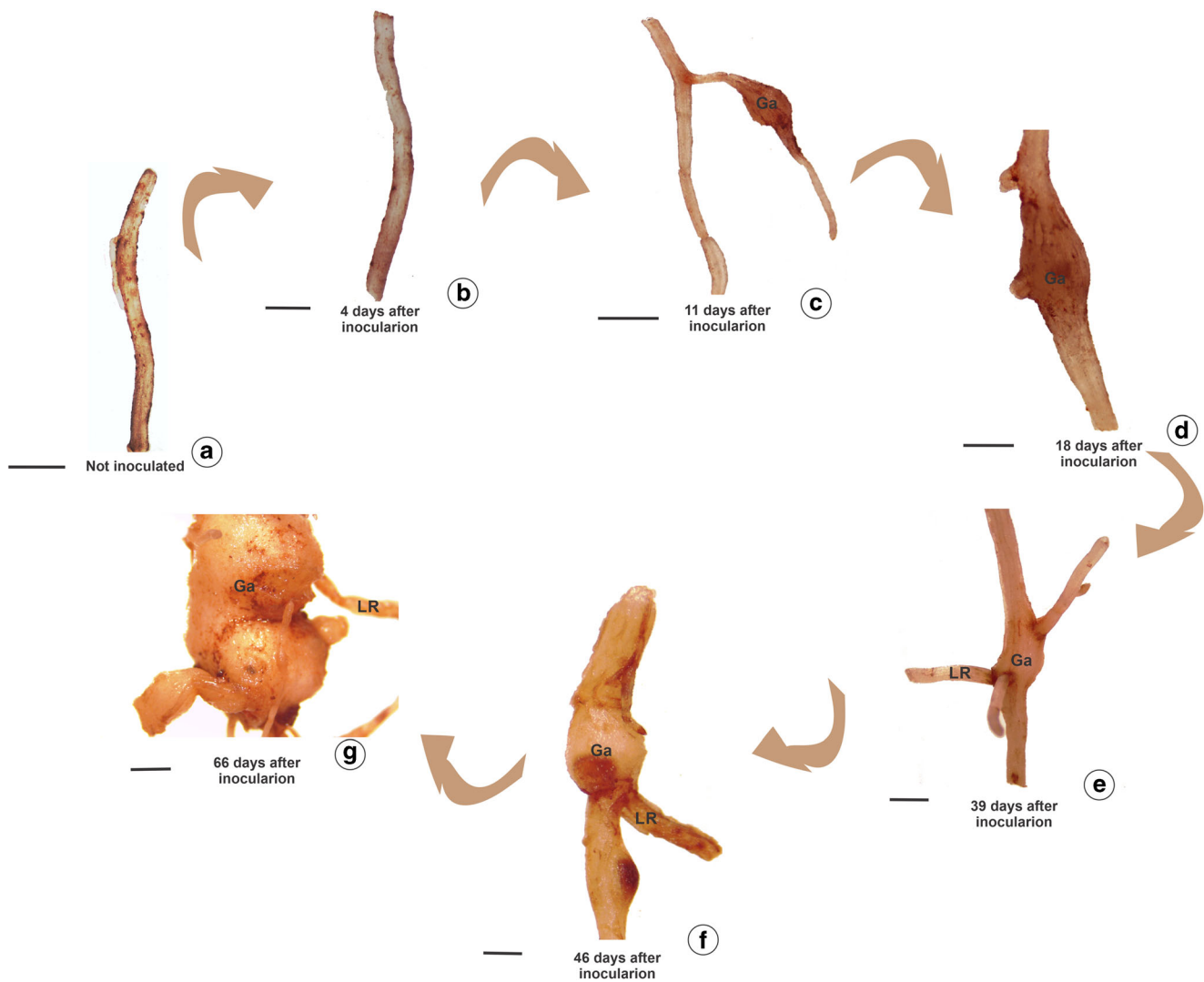


Fig. 2 Morphological changes of okra roots 66 days after inoculation (DAI) of *Meloidogyne incognita* root-knot nematode. **a** Non-inoculated root; **b** 4 DAI, with no morphological signal of galls; **c, d** after 11 and 18 DAI, respectively, with the beginning of root thickening; **e, f** larger and

numerous galls at 39 and 46 DAI, respectively; **g** well-developed and grouped galls of varied shapes and sizes at 66 DAI. *Abbreviations:* Ga = galls, LR = lateral root

a large amount of secondary xylem was produced, leading to a lateral enlargement of the gall (Fig. 3e). A periderm was produced in the outer layers and replaced the epidermis (Fig. 3e). On day 66, new giant cells were produced, with a large increase in the cortical parenchyma through hyperplasia and hypertrophy processes (Fig. 3f, g).

Immunocytochemical analyses

The inoculated and non-inoculated *A. esculentus* roots showed similar results in immunocytochemical analysis (Table 2), which was based on the means and standard deviation present in the [supplementary table](#). However, epitopes of (1→5) α -L-arabinans, recognized by LM6, occurred only in non-inoculated roots (Table 2). These epitopes were intensely labeled in the cortical parenchyma cell walls and moderately in

the parenchymal cell wall of xylem, phloem, and vascular cambium (Table 2; Fig. 4a, b). The epitopes of extensin and (1→4) β -D-galactan, recognized by LM1 and LM5, respectively, were not detected in either treatment (Table 2). Epitopes of HGs with high methyl-esterified groups, recognized by JIM7, were intensely to moderately labeled in the cortical parenchyma cell walls and parenchyma cell walls of xylem in both treatments, as well as in giant cells (Table 2; Fig. 4c, d; Fig. 5a, b). Epitopes of HGs with low methyl-esterified groups, recognized by JIM5, were distributed at the same sites as those detected by JIM7, with differences in intensity (Table 2). Epitopes recognized by JIM5 were intensely labeled in the cortical parenchyma cell wall of the non-inoculated root (Table 2; Fig. 4e, f) and in the parenchyma cell wall of xylem and giant cells of inoculated roots (Table 2; Fig. 5c, d).

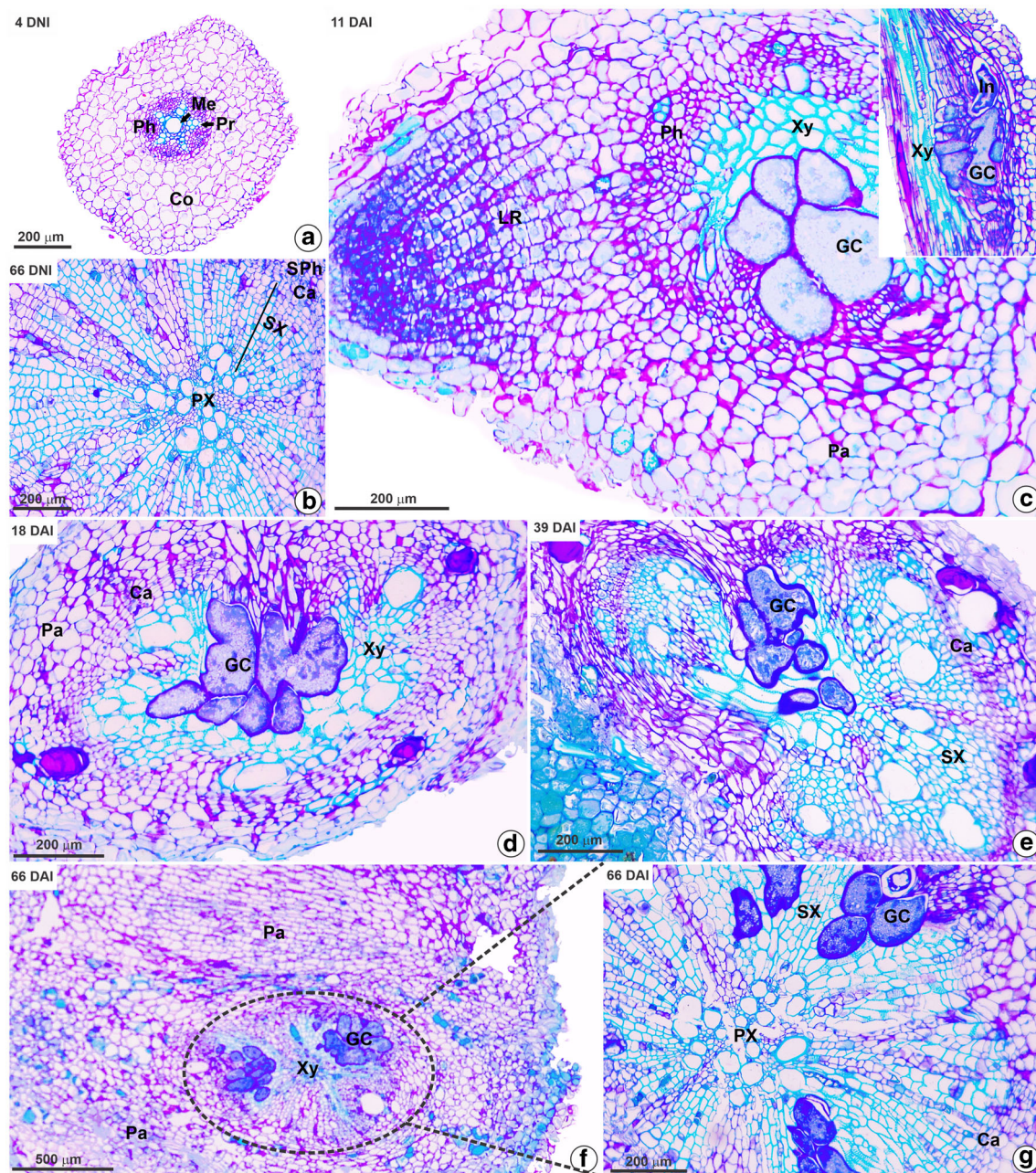


Fig. 3 Histological features of okra roots 66 days after non-inoculated treatment (DNI) and after different days of inoculation (DAI) with *Meloidogyne incognita* root-knot nematodes. **a, b** Non-inoculated roots. **c–g** Inoculated roots. **a** 4 DNI with primary axial root and pentarca organization; **b** 66 DNI with usual secondary growth; **c** 11 DAI in primary growth and giant cells in formation. In detail, note the presence of the female nematode in touch with the giant cells; **d, e** 18 and 39 DAI with

installation of the vascular cambium and the beginning of secondary growth; **f** 66 DAI with larger numbers of giant cells and expanded cortical parenchyma. *Abbreviations:* Ca = vascular cambium, GC = giant cell, Ph = phloem, In inductor, Me metaxylem, Pr protoxylem, Co cortical parenchyma, SPh secondary phloem, SX secondary xylem, PX primary xylem, Pa parenchyma, Xy xylem

Discussion

The root-knot nematode *M. incognita* impacts some agronomic traits of okra, decreasing, for example, the dry weight of the stem system. However, some traits such as the number and weight of fruits increased unexpectedly, with these changes possibly being related to an increase in the production of

lateral roots stimulated by the presence of galls. Giant cells developed from day 11 after inoculation and increased in number and size until 66 days after inoculation. Giant cells came from the parenchyma cells of xylem and showed intense and moderate labeling for epitopes recognized by JIM5 and JIM7. The presence of these HGs with different degrees of methyl esterification seems to be related to the injuries caused

Table 2 Reaction intensity of the epitopes for pectins and proteins of okra roots at 66 days after inoculation of *Meloidogyne incognita* root-knot nematodes (DAI) and non-inoculation (control)

Monoclonal antibodies	Fluorescence intensity												
	Non-inoculated root						Inoculated root						
	Pe	Co	Xy		Ph	VC	Pe	Co	Xy		Ph	VC	GC
			PC	VE					PC	VE			
JIM 5	-	+++	+	-	++	+	-	++	+++	-	+	+	+++
JIM 7	-	+++	+++	-	+	+	-	++	+++	-	+	+	++
LM1	-	-	-	-	-	-	-	-	-	-	-	-	-
LM5	-	-	-	-	-	-	-	-	-	-	-	-	-
LM6	-	+++	++	-	++	++	-	-	-	-	-	-	-

Abbreviations: Pe periderm; Co cortical parenchyma; Xy xylem; PC parenchymal cell of xylem; VE vessel element; Ph phloem; VC vascular cambium; GC giant cell

*Intensity of reaction: (-) Negative (= 0 Gy value); (+) Weak (<15 Gy value); (++) Moderate (15_30 Gy value); (+++) Intense (>30 Gy value)

by the nematode feeding activity, as well as to the typical hypertrophy process that occurs in giant cells (see Vilela et al. 2019).

Nematode infection determines agronomic traits

Okra is a semi-woody annual shrub that can reach 3 m in height; however, the Santa Cruz 47® cultivar is shorter, reaching up to 2 m on average, a height that facilitates harvest (Filgueira 2008). In the present study, okra ranged from 50 to 70 cm in height at harvesting, with a difference between treatments. Unexpectedly, inoculated plants were taller than non-inoculated ones, a result probably due to the moderate stress caused by the nematodes, as already described in the literature (Abrão and Mazzafera 2001). Nevertheless, the nematode-infected plants usually showed a weaker and shorter development of stem systems (Silva et al. 2006). *Meloidogyne* spp. infection can lead to root cortex detachment, interruption of root-tip growth, and extensive mutilation of the root system (Brass et al. 2008), something not detected until 66 days after inoculation in our study.

Fresh root weight did not differ between the non-inoculated and inoculated groups, as also observed in parasitic infection promoted by *M. javanica* in *Tectona grandis* Linn. F. (Oliveira and Silva 2013) and in banana plantations (Cofcewicz et al. 2004). Due to the large number of galls in the inoculated roots in our study, a greater weight of these roots was expected since, according to Carneiro et al. (1999), the formation of galls and the increase in the number and proliferation of lateral roots can lead to an increase in the weight of inoculated plants. For instance, an increase in the fresh weight of the inoculated roots was reported for passion fruit (Sharma et al. 2005). *Meloidogyne* spp. infection also typically reduces plant growth and fruit production

(Ritzinger and Ritzinger 2003), although in our study the inoculated plants, surprisingly, produced more and heavier fruits than non-inoculated plants despite a decrease in the dry weight of the stem system. According to Pinheiro et al. (2013), nematode-caused stress may directly or indirectly influence the performance and survival of okra plants, a fact that was also observed here with *M. incognita* infection of okra.

Okra cultivars are highly prone to root-knot nematode infections and are even used to indicate the presence of *Meloidogyne* spp. (Pinheiro et al. 2013). This expected susceptibility was detected and detailed in the current study. We found over 400 galls per root, including small, medium, and large ones, which, according to the gall index (GI) proposed by Taylor and Sasser (1978), far exceeded the minimum number of 100 for a plant to be considered susceptible. The giant cells are essential for the development of the root-knot nematode, since the nematodes penetrate and establish a feeding site in the root system of plants, thus using their nutrients as food (Kyndt et al. 2013). Galls seem to be good indicators of the presence of the parasite (Davis et al. 2004), with the number of galls in a root permitting the host plant to be classified as susceptible or resistant. Herein, we detected a large number of nematodes in roots and, although the literature suggests that the development and productivity of plants could be affected by the infection, this was observed for okra infection with *M. incognita*, but, unexpectedly, sometimes in a positive way.

Root-knot gall morphology and histopathology

The root-knot nematode induces a root thickening in okra that may be hard to notice before 11 days of inoculation. On the other hand, the gall reaches its maximum size on day 66 after inoculation. Thus, gall formation is the best-known symptom of *Meloidogyne* nematode infection since galls develop just

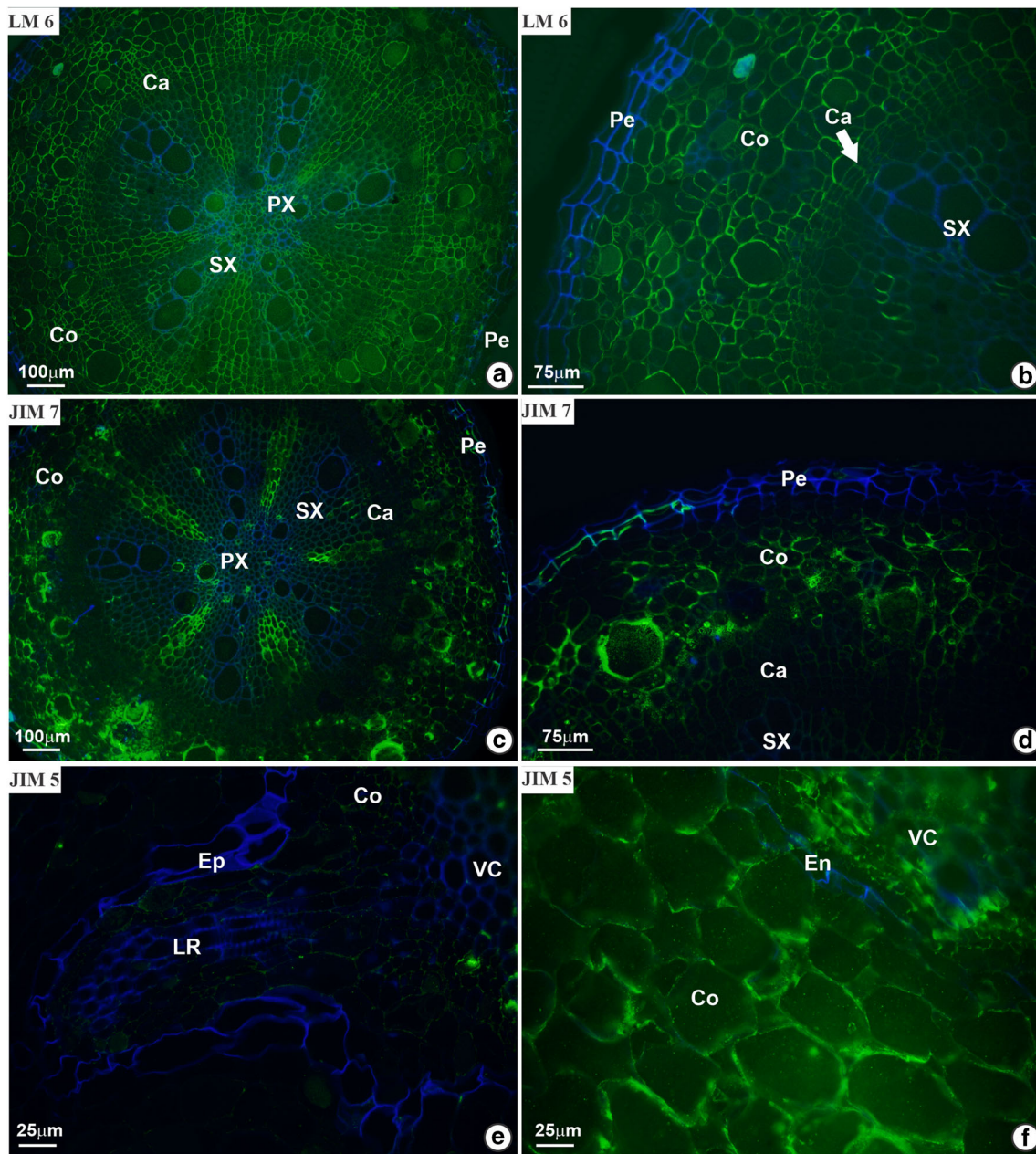


Fig. 4 Immunocytochemistry results for pectic epitopes recognized by LM6 (a, b), JIM7 (c, d) and JIM5 (e, f) antibodies of okra roots at 66 days after non-inoculated treatment (DNI). *Abbreviations:* Ca = vascular cambium, PX primary xylem, SX secondary xylem, Co cortical parenchyma,

Pe periderm, Ep epidermis, LR lateral root, En endodermis. PS.: green labeling on the cell wall indicates a positive result, while blue labeling shows autofluorescence using the DAPI filter

after the juveniles infect the roots (Mitekowski and Abawi 2003). According to these authors, galls have an elongated shape and a swollen appearance and occur throughout the root system, as was the case for the galls observed in our study. These galls are a result of cell hypertrophy of the cortex or of adjacent cells surrounding the nematode, as well as tissue hyperplasia usually of the pericycle (Vilela et al. 2019). The development of root-knot nematode galls may be the consequence of secretions from esophageal glands injected by the buccal stylet of the nematode, resulting in the formation of

giant cells and of galls. The two structure formations are independent of each other (Ferraz and Brown 2016) and, sometimes, galls may be small or indistinct (Moens et al. 2009) or may be absent (Davis et al. 2004; Ferraz and Monteiro 2011).

Herein, we first noted the formation of a feeding site with giant cells on day 11 after inoculation, differently from the findings of Vilela et al. (2019), who noticed giant cells induced on soybean by *M. javanica* on day 18 after inoculation. Also, root-knot galls induced by *M. graminicola* and *M. incognita* on rice (*Oryza sativa* L.) showed different times

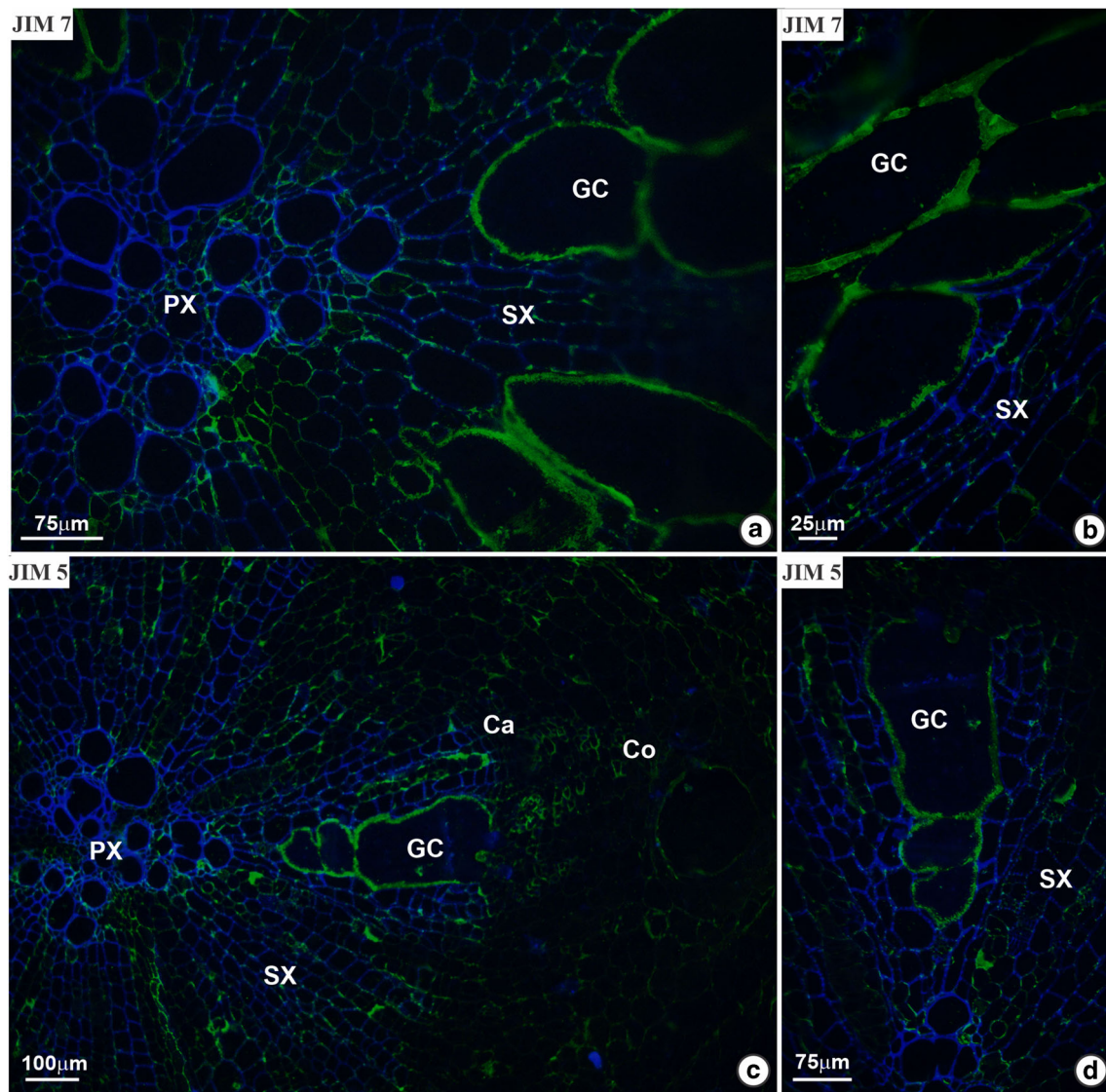


Fig. 5 Immunocytochemistry results for pectic epitopes recognized by JIM7 (**a, b**) and JIM5 (**c, d**) antibodies of okra roots at 66 days after inoculation (DAI) with *Meloidogyne incognita* root-knot nematodes. *Abbreviations:* Ca = vascular cambium, PX primary xylem, SX

secondary xylem, Co cortical parenchyma, GC giant cell. PS.: green labeling on the cell wall indicates a positive result, while blue labeling shows negative results using the DAPI filter

of giant cell formation, which developed 2 and 6 days, respectively, after inoculation (Nguyen et al. 2014). Giant cells are essential for the development and reproduction of the parasite since they act as a metabolic drain of the plant, taking nutrients from the host plant organs to feed the nematodes (Galbieri and Belot 2016). These worms do not kill host cells (Abad et al. 2003), but rather form a feeding tube (Davis et al. 2004) from the secretions of the esophageal glands towards the cytoplasm of the plant cell, filtering the cytosol during ingestion (Mitkowski and Abawi 2003). Giant cells typically have a dense cytoplasm, numerous nuclei, and thick cell walls, as observed by Castañeda (2015) in an investigation of banana roots (*Musa acuminata* subgroup Cavendish, cv. Grande Naine) infested with *M. incognita*. Using electron microscopy to study *Glycine max*

root galls induced by *M. javanica*, Vilela et al. (2019) revealed that the walls of giant cells were thicker and exhibited invaginations towards the cell lumen. The cell walls undergo thickening and loosening processes to expand and support the absorption of nutrients by the nematodes (Escobar et al. 2015; Bohlmann and Sobczak 2014). The presence of giant cells at 11 days after okra inoculation with *M. incognita* disorganized the central vascular cylinder of the roots and could compromise water transport and nutrient uptake, leading to plant wilt, yellowing, and reduced growth (Dorhout et al. 1991; Anwar and Javed 2010; Premachandra and Gowen 2015). Herein, based on agronomic traits, the growth of the okra plants was not compromised even though giant cells and gall formation disorganized the histological profile of the roots.

Cell wall composition of parenchyma cells of xylem and giant cells

In general, galls develop from tissue hyperplasia and cellular hypertrophy (Oliveira et al. 2016), and these processes are dependent on changes in cell wall dynamics and composition, as discussed for insect galls (Oliveira et al. 2014; Carneiro et al. 2015; Teixeira et al. 2018; Martini et al. 2019). The development of root-knot nematode galls also requires a sequence of destructive and constructive cell wall modifications (Bohlmann and Sobczak 2014), especially driven by the action of different pectolytic enzymes (see Wieczorek 2015). The detection of pectic epitopes by monoclonal antibodies has been used as a tool to detect pectolytic activities in gall tissues (Oliveira et al. 2014). Herein, cell hypertrophy was a striking feature of the root cortex, leading to gall formation on okra induced by *M. incognita*. Giant cell development also requires changes in the distribution and dynamics of epitopes of high and low methyl-esterified HGs.

HGs with high methyl-esterified groups were intensely detected in the parenchyma cells of xylem in both non-inoculated and inoculated plants. Giant cells showed moderate labeling for HG epitopes with a high degree of methyl-esterification since these cells come from the parenchyma cells of xylem. HGs are linear homopolymers with approximately 100 molecules of (1-4)- α -linked-D-galacturonic acid, synthesized in the Golgi apparatus in a high methyl-esterified form (Albersheim et al. 2011). The presence of HGs with high methyl-esterified groups is usually detected on young tissues during the processes of growth and elongation (Knox 1992; Albersheim et al. 2011; Wolf and Greiner 2012) and, in galls, it has been associated with the processes of cell hypertrophy during development (Carneiro et al. 2015; Oliveira et al. 2016; Teixeira et al. 2018; Martini et al. 2019). The presence of epitopes with high methyl-esterified groups in the cell walls of giant cells may guarantee cell growth and elongation and, therefore, the establishment of root-knot nematodes.

HGs with low methyl-esterified groups were weakly detected in the parenchyma cells of xylem in non-inoculated plants and intensely in inoculated ones and also detected intensely in the giant cells. The process of HG de-methyl-esterification depends on the action of pectin methyl-esterases (PME) or polygalacturonases (PG) (Willats et al. 2000; Hongo et al. 2012). Since the random de-methyl-esterification of HGs leads to whole cell wall degradation and cell death (Hongo et al. 2012), we suppose that these processes in the root-knot nematode gall on okra occur by the action of PMEs. Our results support an intense process of synthesis and de-methyl-esterification of parenchyma cell walls of xylem and giant cells on the roots of inoculated plants. The de-methyl-esterification process and the presence of low methyl-esterified epitopes of HG in the parenchyma cells of xylem and giant cells may increase cell stiffening and porosity, as

already detected in galls induced by insects (Oliveira et al. 2014; Carneiro et al. 2015; Martini et al. 2019). Herein, the increase in porosity may have favored the nutrient exchange between host tissues and nematode galls induced by *M. incognita* in okra.

Conclusions

The root-knot nematode *Meloidogyne incognita* induces galls and giant cells on the roots of okra, disorganizing the histological profile of root tissues. However, the disorganization of the anatomical structure of the root did not affect the growth and development of okra until 66 days after inoculation; on the contrary, the inoculated plants even grew more. Giant cell formation occurs at 11 days after inoculation and is essential for the establishment of root-knot nematodes. As a feeding site for the root-knot nematode, giant cell formation depends on changes in the degree of HG methyl-esterification of the xylem parenchyma cell wall in order to elongate and in nutrients flux due to increased porosity.

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Availability of data and material All data generated or analyzed during this study are included in this published article.

Authors' contributions DCO and RMIFV conceived and designed the research. RMIFV, CAM, and ACPF conducted the cultivation experiments and collected the agricultural traits. RMIFV, VCK, and DCO conducted the immunocytochemistry experiments. All the authors wrote the manuscript.

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Declarations

Competing interests The authors declare that they have no competing interests.

Consent to participate All authors agree with the participation.

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