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"The false root-knot nematode: Modification of the root anatomy and alteration of the physiological performance in tomato plants"

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ABSTRACT

This work relates the biological cycle of the plant-parasitic nematode *Nacobbus aberrans* and its impact on the morphology and physiology of tomato (*Solanum lycopersicum* L) plants. Tomato var. platense plants were grown in 10 L pots in a greenhouse using a previously sterilized substrate. Half of the plants were inoculated at the time of transplanting with 5000 eggs of the nematode (parasitized treatment) and the other half remained non-inoculated (control). Histological sections performed 75 days after inoculation showed that the establishment of adult females of *N. aberrans* in roots caused a displacement of the xylem and phloem. This alteration induced a series of changes and symptoms in parasitized plants compared to control plants. Stomatic conductance, photosystem II efficiency, and CO_2 fixation (net photosynthesis) showed significantly lower values in parasitized plants. Leaf chlorophyll content and soluble protein content were also reduced. Plants inoculated with the nematode showed a higher accumulation of osmoregulatory metabolites such as proline and sugars as well as malondialdehyde, an indicator of cell membrane damage. Results indicate that the symptoms and alterations caused by this phytoparasite are consistent with those of a plant subjected to water stress.

1. Introduction

Nacobbus aberrans, commonly known as false root-knot nematode, named so because it causes a symptomatology similar to the one caused by the root-knot nematode *Meloidogyne incognita*. This nematode causes important yield losses in horticultural crops. Its presence and damage have been reported in Peru, Bolivia, United States, Chile, Argentina, Mexico, and Ecuador (Manzanilla-López et al., 2002). In Brazil, Israel, Russia, and the European Union, it is considered a quarantine pest, ranked in all cases in the highest danger category because of its potential to cause serious economic damage (EPPO, 2019). In the last decade, *N. aberrans* was included in the list of the 10 most important nematode species globally, due to the economic damage it causes and the research conducted on it (Jones et al., 2013). Much of the recent research on this species is focused on the search for tools that control the population (Cortez-Hernández et al., 2019; Sosa et al., 2018) or on phylogenetic studies aimed at identifying different species within the *Nacobbus* genus (Lax et al., 2021; Cabrera-Hidalgo et al., 2019), with few studies analyzing its interaction with plants.

In general, symptoms on the aerial part of attacked plants include reduced and stunted growth, chlorosis, rolling of leaf margins, wilting, and reduced fruit size (Doucet and Lax, 2015).

In the roots, the main visible symptom is the presence of galls and alterations in the growth of secondary and tertiary ramifications. Sometimes the roots can be totally atrophied or necrotic due to the combination of the attack of the nematode with that of other pathogenic microorganisms (Schuster et al., 1965).

The life cycle of *N. aberrans* consists of an egg stage, followed by four larval or juvenile stages, and an adult stage. Inside the egg, the first juvenile stage (vermiform in appearance) is formed; the first molt also occurs there to give rise to the second juvenile stage (J2) (Clark, 1967). Under suitable conditions, J2 will emerge to the soil, enter the root, and emerge repeatedly during molting (Juvenil second and third stages) until it reaches the adult stage (Manzanilla-López et al., 2002).

Adult males are vermiform and can be found inside roots or free in the soil. females attach themselves in the vicinity of the central cylinder,

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where they induce the formation of their feeding site called syncytium. These females lose their filiform shape and become bulky due to the development of the eggs inside them (Manzanilla-López, 1997). The growth in volume of females and the formation of the syncytium result in the formation of galls, which are the most characteristic visible symptom of nodulating nematodes.

This work relates the life cycle of the nematode *N. aberrans* and its parasitism mechanisms to the morphological damage it causes in root tissues and quantifies its consequences on the physiological performance of 'Platense' tomato plants in the absence of other pathogenic microorganisms.

2. Materials and methods

2.1. Test conditions

Twenty seedlings of tomato (*Solanum lycopersicum*) var. Platense with four expanded leaves were transplanted into 15 L pots containing as substrate argiudol soil previously tindalized (by three heating cycles of 1 h at 100 °C on consecutive days) to eliminate possible pathogenic microorganisms and evaluate only the effect of the nematode. The pots were placed in a greenhouse (34°54′45.5 "S 57°55′51.5 "W) with natural lighting, forced ventilation, and controlled temperature. The day after transplanting, half of the plants were inoculated with 5000 eggs of *N. aberrans*, and two treatments were established: noninoculated control plants and inoculated plants. Inoculation was carried out by making two holes in the substrate around the plant, depositing 2.5 mL of the egg suspension used as inoculum in each hole and then covering the holes with the same substrate. The egg suspension was obtained using the technique of shaking the roots in 0.5% NaClO solution (Hussey and Barker, 1973) with subsequent purification and concentration by the centrifugation-flotation technique (Coolen, 1979).

Morphological, biochemical, and physiological determinations were carried out 21 days after transplanting (only in aerial tissue samples) and repeated 75 days after transplanting (in aerial and root tissue). During the course of the test, mature fruits were harvested from each plant.

Evaluated parameters.

- Plant height.
- Stem diameter measured at the middle of the plant.
- Net photosynthesis (NP) using an IRGA portable infrared gas analyzer (model CIRAS-2®, PP Systems), average temperature 25 °C, CO_2: 360 ppm, photon flux:1500 μmol m-$^{2s-1}$.
- Stomatal conductance (gs); Idem above.
- Quantum yield of photosystem II (PSII), using a fluorescence detector (Hansatech Instruments, UK, type FM52); results were analyzed according to Genty et al. (1989).
- Greenness index, Minolta SPAD 502.
- Total chlorophyll and carotenoid content (leaf tissue only) (Wellburn, 1994).
- Soluble protein content (Bradford, 1976). The protein concentration was calculated using a standard curve prepared with different concentrations of bovine serum albumin (BSA, SiFMa Chemical Co).
- Proline content (Bates et al., 1973).
- Malondialdehyde content (Heath and Packer, 1968). MDA concentration was calculated using an extinction coefficient of 155 mM^{-1} cm^{-1}.
- Content of total phenolic compounds according to the method based on the use of the Folin-Ciocalteu reagent.
- Content of reducing and total sugars (Cronin and Smith, 1979).
- Relative tissue conductivity (Lutts et al., 1996).
- Histological sections and staining of roots to characterize histopathological modifications of root tissue (Zarlavsky, 2014; Gurr, 1971).
- Number of eggs lodged in roots, number of juveniles in soil, and final nematode population (Coolen,1979; Hussey, R. S. and Barker, K. R. 1973).
- Fruit weight and percentage of defective fruit.

2.2. Experimental design and statistical analysis

The test had a completely randomized design with two treatments and 10 replicates per treatment. Each plant corresponded to one replicate. The treatments consisted of "noninoculated plants" and "plants inoculated with *N. aberrans*". The analysis of the results was performed by means of a one-factor analysis of variance; the comparison of means was done by Fisher's LSD test using the statistical program InfoStat.

3. Results

No significant differences were observed between control and inoculated plants ($p \geq 0.05$) in the growth, biochemical, and physiological parameters evaluated 21 days after inoculation (Table 1).

When the roots were washed 75 days after inoculation, the presence of numerous galls could be observed in the treatment inoculated with *N. aberrans*, confirming that the inoculation was effective. Egg extraction and quantification indicated an average value of 90,060 eggs per plant.

Histological sections of parasitized roots showed that the secondary structure of the vascular tissue was altered with respect to the structure of healthy roots (Fig. 1). The secondary phloem ring was deformed, as well as the secondary xylem. The presence of parenchyma between the phloem and xylem and in the xylem itself was observed. In the cortex, cellular disorder and increased parenchyma layers were manifested. In the cortical parenchyma, abundant starch grains and perforations were observed. The dark spots correspond to crystalline sands typical of the *Solanaceae* family.

Leaf surface gas exchange, carbon dioxide fixation, and photosystem II efficiency were significantly higher in nonparasitized plants (Table 2).

Means and standard deviations followed by different letters indicate

Table 1
Analysis of variance, mean values, and standard deviation of growth, biochemical, and physiological parameters measured after 21 days in tomato Platense plants parasitized and nonparasitized (control) with *Nacobbus aberrans*.

Parameter	CV	*p*-value	F	Control	Inoculated
Plant height (cm)	11.95%	0.79	0.07	29.7 ± 2.8	30.1 ± 3.11
Stem diameter (cm)	17.7%	0.15	2.42	0.58 ± 0.1	0.62 ± 0.1
Stomatal conductance ($mmol.m^{-2}s^{-1}$)	17.67%	0.72	0.13	62.48 ± 7.2	64.73 ± 8.4
PSII efficiency (Fv/Fm)	14.11%	0.96	0.002	0.34 ± 0.05	0.33 ± 0.04
Total chlorophyll (μg. cm^{-2})	9.68%	0.11	2.94	35.17 ± 2.8	38.71 ± 2.7
Total carotenoids (μg. cm^{-2})	13.38%	0.09	12.02	5.19 ± 0.49	6.79 ± 1.02
Soluble proteins (μg.mg FW^{-1})	17.8%	0.77	0.09	3.7 ± 0.51	3.59 ± 0.77
Relative conductivity (%)	4.81%	0.80	0.07	97.42 ± 6.16	98.12 ± 2.51
Phenols (μg. mg PF^{-1})	12.58%	0.91	0.01	1.06 ± 0.1	0.97 ± 0.09
Malondialdehyde (nmoles.gr FW^{-1})	20.2%	0.35	0.93	12.79 ± 2.94	11.42 ± 3.09
Proline (μmoles.g FW^{-1})	14.39%	0.53	0.41	317.11 ± 68.7	300.75 ± 70.7
Total sugars (μg. g FW^{-1})	26.6%	0.32	1.09	403.2 ± 98.08	473.6 ± 133.2

CV: coefficient of variation; *p*-value: probability; F: F calculated; FW: fresh weight.

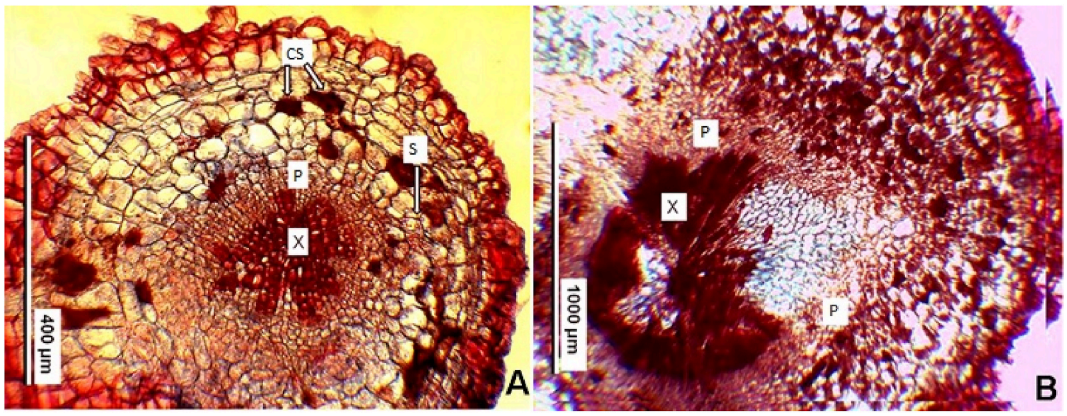

Fig. 1. Cross section of: a) root without galls; b) root at the level of a gall. CS: crystalline sand, S: starch, P: secondary phloem ring, X: secondary xylem.

Table 2
Physiological performance of tomato Platense plants parasitized and non-parasitized (control) with Nacobbus aberrans.

	CV	Control	Parasitized
Stomatal conductance (mmol. $m^{-2}s^{-1}$)	26.4%	160.2 ± 53.5 A	94.4 ± 17.7 B
Quantum yield of PSII (Fv/Fm)	21.1%	0.55 ± 0.17 A	0.24 ± 0.08 B
Net photosynthesis (mmol.$m^{-2}s^{-1}$)	7.43%	18.60 ± 2.77 A	10.86 ± 1.67 B
Stem diameter (cm)	14.71%	1.00 ± 0.1A	0.70 ± 0.11 B
Weight of harvested fruits (g)	18.49%	1044 ± 241 A	1131 ± 149 A
Average weight of harvested fruits (g)	24.55%	133.8 ± 32.6 A	134.5 ± 33.2 A
Percentage of defective fruits (%) *	16.2%	8.2 ± 4.1 B	38.4 ± 7.3 A

significant differences according to Fisher's LSD test ($p \leq 0.01$). * For the statistical analysis, an arcsine transformation of the values was performed CV: coefficient of variation.

During the course of the test, five fortnightly height measurements were made and showed no significant differences between treatments (data not shown). However, there were significant differences in stem diameter measured at mid-plant height (Table 2).

Regarding harvested fruits, there were neither significant differences in the total weight of harvested fruits nor in the average weight of harvested fruits. However, there were significant differences in fruit quality. Plants parasitized by *N. aberrans* had a higher number of fruits with cracking and deformed fruits (Table 2; Fig. 2).

Determinations made with Spad to measure leaf greenness index showed significant differences (p 0.01, CV: 11.77%) between non-inoculated and inoculated plants with *N. aberrans*. Likewise, the determination of total chlorophyll by spectrophotometry confirmed this with significant differences between the concentrations measured for the two treatments ($p \leq 0.01$, CV: 10.31%) (Fig. 3).

Columns accompanied by different letters indicate significant differences according to Fisher's LSD test ($P \leq 0.05$).

The determination of soluble protein content by Bradford's method (1976) also showed significant differences between treatments, both in leaves and roots (Fig. 4).

Determinations of reducing sugars in leaf and root tissue samples showed no significant differences between treatments (Fig. 5), whereas the concentration of total sugars in leaves and roots were significantly higher in parasitized plants (Fig. 5).

Stress-related determinations in plants such as proline content,

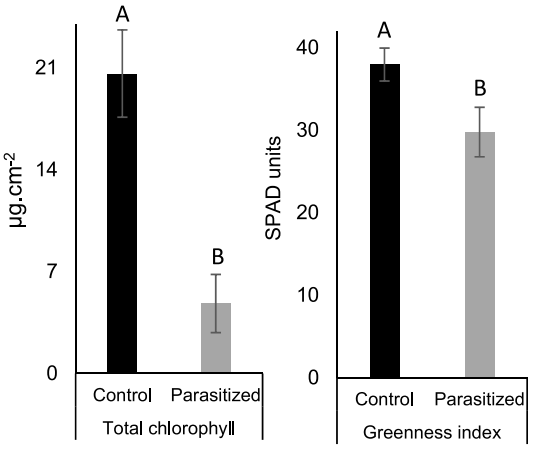

Fig. 3. Greenness index and total chlorophyll content in tomato plants parasitized and nonparasitized (control) with *Nacobbus aberrans*.

Fig. 2. Tomato Platense fruits: a) normal; b) cracked, and c) deformed.

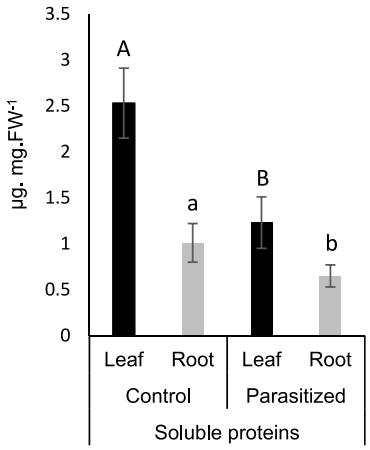

Fig. 4. Soluble protein content in leaf and root tissue of tomato Platense plants parasitized and nonparasitized (control) with *Nacobbus aberrans*. Uppercase letters compare leaves; lowercase letters compare roots. Columns accompanied by different letters indicate significant differences according to Fisher's LSD test ($P \leq 0.01$).

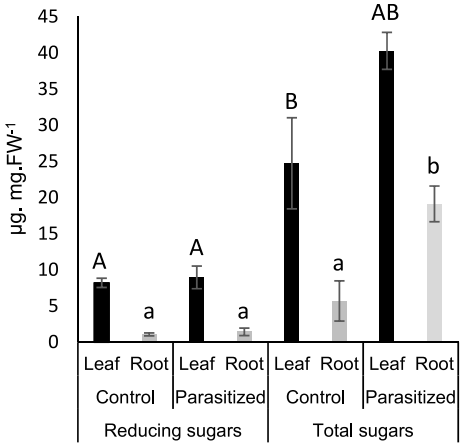

Fig. 5. Content of reducing and total sugars in leaf and root tissue of tomato Platense plants parasitized and nonparasitized (control) with *Nacobbus aberrans*. Uppercase letters compare leaves; lowercase letters compare roots. Columns accompanied by different letters indicate significant differences according to Fisher's LSD test ($P \leq 0.05$).

Table 3
Proline content, malondialdehyde, and relative conductivity in leaf and root tissue of tomato Platense plants parasitized and nonparasitized (control) with *Nacobbus aberrans*.

Parameter	CV	Control	Parasitized
Proline leaf tissue µmoles.g FW^{-1}	18.02%	740.8 ± 180.7 A	777.3 ± 68.9 A
Proline root tissue µmoles.g FW^{-1}	11.4%	379.5 ± 54 B	1188.8 ± 115.1 A
MDA leaf tissue nmoles.gr FW^{-1}	22.3%	1.2 ± 0.26 A	1.61 ± 0.36 A
MDA root tissue nmoles.gr FW^{-1}	15.61%	6.44 ± 1.1 B	10.42 ± 1.5 A
Leaf relative conductivity (%)	28.3%	23.75 ± 5.1A	35.22 ± 9.7 A
Root relative conductivity (%)	13.79%	6.44 ± 1.3B	10.42 ± 1.8 A
Phenols leaf tissue (µg. mg FW^{-1})	19.22%	1.36 ± 0.29 A	1.40 ± 0.23 A
Phenols root tissue (µg. mg FW^{-1})	15.04%	0.21 ± 0.03 b	0.99 ± 0.13 a

Means followed by different letters indicate significant differences according to Fisher's LSD test ($P \leq 0.05$).

malondialdehyde content, and relative electrical conductivity showed no differences in leaf tissue. In root tissue, these determinations showed significant differences between parasitized and control plants (Table 3). The quantification of phenols in leaf tissue showed no differences between control and parasitized plants; however, in root tissue the differences were significant ($p \leq 0.01$, CV: 15.04%) (Table 3).

4. Discussion

In the different measurements and determinations performed 21 days after inoculation, no significant differences were observed between parasitized and nonparasitized plants. Although the time elapsed was sufficient for *N. aberrans* to carry out at least one complete life cycle, it is estimated that the increase in the population was not sufficient to exert a detrimental effect on the plants.

As the test progressed, successive life cycles of the nematode occurred, causing an increase in its population. The entrance and movement of *N. aberrans* in the root caused perforations and cell death. However, the main alteration in the root is due to the sedentary stage of adult females that displace the conduction tissues, which in turn modifies the normal flow of water. These statements are based on what was observed in histological sections and stomatal conductance measurements, where the values measured in parasitized plants were significantly lower than in control plants. One of the fastest and most common responses of plants to limit water vapor loss by transpiration is the partial or total closure of stomata. Consequently, water loss decreases, but so does the flow of CO_2 in the direction of the mesophyll and thus the photosynthesis (Bosco et al., 2009).

Out of the total light energy absorbed by chlorophylls and carotenoids, one part is used to carry out the photosynthetic process, another part is dissipated as heat and another part is re-emitted as longer wavelength light energy (fluorescence). These three events occur simultaneously, and by knowing the value of the energy emitted by fluorescence, it is possible to infer the efficiency of the system. Transpiration is the main mechanism for dissipation of heat energy from the leaves, so if this regulatory pathway is affected or diminished, the plant is forced to emit more energy through fluorescence.

When irradiance is normal or high, stomatal closure can cause excitation of photoreceptors that leads to the formation of reactive oxygen species (ROS) that damage the photosystem (Ghobadi et al., 2013). The results demonstrate that the differences observed in net photosynthesis were not only due to stomatal closure, but that the photosynthetic potential of the control plants was higher because of a higher content of photosynthetic pigments.

Some authors cite that a common response of plants that suffer root damage is a rapid reduction in protein concentration (Bray 1997; Zhu et al., 2002). This reduction is due to a proteolysis mechanism that occurs in order to elevate the concentration of solutes (amino acids) in the cytosol and decrease the osmotic potential, thus increasing the possibility of water and nutrient absorption (Javot, 2002).

Solutes such as proline and low molecular weight sugars accumulate in tissues in response to water deficits to decrease the osmotic potential of the cells and thus favor the absorption and movement of water in the plant. According to the obtained results, nonreducing sugars play the most important role in osmotic adjustment, which is in agreement with the conclusions reached by Herrera Flores et al. (2012) in studies of osmotic adjustment and water stress.

Fruit cracking is a physiological alteration in which the epidermis cracks at different depths (Pascual et al., 1998). The greatest cracking in the fruits of parasitized plants must have been associated to water balance disorders (Osvald and Osvald, 1991), higher plant temperature as a consequence of water stress, and difficulties in the translocation of calcium that alters membrane flexibility (Peet, 1992).

Phenolic compounds are a very broad group of molecules; in this analysis, only total values have been quantified, without knowing in detail which type of phenolics is predominant. However, it may be

stated that they are post-infectional compounds that increased following the attack of the nematode. The antagonistic relationship between phenolic compounds and nematodes has been documented in different ways. Cultivars with higher tolerance or resistance to nematodes exhibit elevated phenol contents in their roots (Banora and Alhakim Almaghrab, 2019).

The damage caused by the nematode begins with the rupture of the cells with the stylet, the dissolution of the wall by the action of enzymes that modify cell physiology (Sijmons, 1993). Malondialdehyde is the main product of peroxidation, and its increase is considered an indicator of oxidative stress and plasma membrane damage. Although the results indicated differences, values do not demonstrate lipid peroxidation as high as that documented in severe stresses of different types (Sharma and Sharma, 2017). Possibly, because of its biotrophic condition, the laceration caused by the nematode on the tissue is limited to its movement in and out of the root. The remaining root alterations modified functionality without causing oxidative stress. Since the substrate is tindalized, the damage observed in the roots is exclusively caused by *N. aberrans* and not by other microorganisms that, under real production conditions, form a nematode-fungus complex that jointly affects the plants more aggressively.

According to the obtained results, the tomato crop affected by *N. aberrans* is consistent with a plant subjected to water stress and nutritional stress, with the resulting alterations. It should be noted that in a production system there are other variables that accentuate these responses, such as the presence of opportunistic pathogens and high greenhouse temperatures that contribute to water stress. In previous researches, these authors have shown that using tools that facilitate the absorption of water and nutrients such as inoculation with mycorrhizal fungi (BernardoGarita et al., 2020) and the use of rootstocks with large root systems (Garita et al., 2018) improve the physiological performance of crops attacked by *N. aberrans*.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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