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ORIGINAL ARTICLE

Comprehensive analysis of Nematode–Tomato Plant–Mycorrhizal Fungus system for bio based product development

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Abstract
Nacobbus aberrans is a plant parasitic nematode that causes significant economic losses in the American Continent and is considered a quarantine pest in many countries. Some arbuscular mycorrhizal fungi have shown the ability to reduce the population of this pathogen; however, most studies do not consider other relevant aspects that have to do with the crop or the fungus. The purpose of this work was to select an isolated mycorrhizal fungus based on a comprehensive analysis of the Nematode–Plant–Mycorrhizal Fungus system. The test was conducted on *Solanum lycopersicum* L. grown in pots. The evaluated isolates were *Funneliformis mosseae*, *Rhizoglomus intraradices A2*, and *Rhizoglomus intraradices B1*. All the isolates reduced the nematode population significantly; however, differences in the content of photosynthetic pigments, soluble proteins, and osmoregulatory metabolites were identified. All of these impacted on the photosynthetic rates of the different treatments. There were also differences in the growth of the fungi within the roots, and in the viability of the fungal structures. The comprehensive analysis of the plant–nematode–fungus group allows us to conclude that the isolate with the greatest capacity to compensate for the negative effect of parasitism, and with the greatest possibility of lasting as a biocontrol agent is *Funneliformis mosseae*.

Keywords *Nacobbus aberrans* · Biocontrol · *Funneliformis mosseae* · Mycorrhiza

Introduction

Nacobbus aberrans is a parasitic nematode of plants that causes great economic losses in the horticultural crops of the American continent. The tomato, *Solanum lycopersicum* L, belongs to the *Solanaceae* family and is the second most important vegetable in the world due to its production volume, cultivated area, and the technology and research developed around it (FAO 2023). Like 84 other botanical species, the tomato is susceptible to attack by *N. aberrans* which can cause yield losses of 50–90% in Latin America (Manzanilla-López et al. 2008).

The juvenile stages of this pathogen are vermiform, have a length of 300–400 µm and can pierce the root tissue with their stylets to feed. Young females enter the radicular cortex, where they remain as sedentary endoparasites for oviposition. At this stage, they cause hyperplasia and cellular hypertrophy giving rise to their feeding sites called syncytia (EPPO 2009). Syncytia cause a disorganization of conductive tissues, altering the normal flow of water and nutrients (Garita et al. 2019). The external manifestation of this process is the presence of galls on roots. The control of this nematode is traditionally carried out with highly toxic soil fumigants, leading to a constant search of biological tools to mitigate this adversity.

The symbiosis between mycorrhizal–arbuscular, in which fungi of the *phylum* Glomeromycota are associated with most of the roots of higher plant species, has been widely studied from different scientific approaches. In addition to intraradicular growth, the hyphae extend in the soil several tens of centimeters thus expanding the depth and extension

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of root exploration which determines various agronomic benefits. The increase in the volume of soil explored by hyphae and the higher absorption rate per unit area puts mycorrhizal plants at an advantage over non-mycorrhizal plants. The external mycelium exudes hydrophobic glycoproteins called glomalin-related soil protein, highly stable carbon-rich compounds that cause drastic increases in soil biological activity and aggregate stabilization (Rillig 2004; Wang et al. 2021). Although mycorrhization does not cause visible modifications in the root structure, metabolic modifications occur that change its relationship with the environment. The synthesis of hydrolytic enzymes, such as chitinase and β–1,3–glucanase (El–Khallal 2007), and enzymes associated with the synthesis of phenolic compounds (Guillon et al. 2002) is modified. The above information about mycorrhizal symbiosis allows us to explain the existence of innumerable investigations interested in evaluating how mycorrhized tomato plants can more easily tolerate or overcome different stress situations: low temperatures, flooding, drought (Ruscitti et al. 2015); salinity (Abdel Latef and Chaoxing 2011); heavy metal toxicity (Cavagnaro et al. 2009); and extreme temperatures (Morales-Guevara et al. 2018). There also have been documented the benefits against fungal pathogens (Pérez Ortega et al. 2015); bacterial (Abo-Elyousr et al. 2014; Tahat et al. 2012); chewing insects (Song et al. 2013); and nematodes (Garita et al. 2019).

This work proposes to evaluate isolates of arbuscular mycorrhizae-forming fungi for the control of *N. aberrans* with a comprehensive analysis, analyzing biochemical and physiological parameters of the host plant (*Solanum lycopersicum* L.), parameters linked to the population of the pathogen, and parameters of the mycorrhizae forming fungus.

Materials and methods

Plant material and inoculation with Arbuscular mycorrhizal fungi (AMF)

To produce the seedlings of the test, tomato seeds var. platense were disinfested with NaOCl (10%) for 5 min and were planted in 4 seedling trays of 72 cells each, with a capacity of 5.5 mL each. The mixture used to complete the cells was formed by autoclaved perlite, vermiculite, and mycorrhizae inoculum, in a ratio of 1:1:1. The inoculum contains one of the following fungi: *Funneliformis mosseae* (Fm), *Rhizoglomus intraradices* B1 (Ri–B1), or *Rhizoglomus intraradices* A2 (Ri–A2), and autoclaved inoculum to be used in the control treatment. The provision of nutrients was made using a complete nutrient Hoagland solution. The inocula used consist of a mixture of perlite, vermiculite, and ground roots, which have a concentration of 100 spores g^{-1}.

The isolates of the genus *Rhizoglomus* come from the "Bank of Glomeromycota in–vitro" (BGIV) Buenos Aires, Argentina. And the isolate of the genus *Funneliformis* comes from the "Instituto Spegazzini" (LPS) La Plata, Argentina.

After 35 days of planting, 5 tomato seedlings were removed from each tray; the roots were washed, and the technique described by Phillips and Hayman (1970) was applied to dye the roots. Preparations were then made with the roots to confirm and quantify the level of mycorrhization (Trouvelot et al. 1986).

Transplant

At 40 days after sowing, the transplant was carried out to 10 L pots, using tindalized soil as substrate (Vertic Argiudol, pH 5.5, 12 mg kg − 1 total P, 3.5% organic matter, 2% total C, and 0.24% total N). The sterilization was performed to eliminate the positive or negative effect of any other soil microorganism and to visualize exclusively the action of mycorrhizae against *N. aberrans*. The plants were placed in a greenhouse with forced ventilation, the temperature during the test was maintained between 22 and 29 °C, natural lighting with average irradiance of 5.75 Kw.h m^{-2}, and irrigation was carried out daily.

Inoculation with *N. aberrans*

The suspension with eggs and juveniles used as inoculum was obtained from greenhouse-maintained tomato plants whose roots host a monoxenic population of *N. aberrans*. The extraction was carried out using the technique of shaking the roots in a 0.5% NaClO solution (Hussey and Barker 1973) with subsequent purification and concentration by the centrifugation–flotation technique (Coolen 1979). The day after the transplant half of the plants were inoculated with 5000 eggs and juveniles of *N. aberrans*, defining the different treatments.

Non-mycorrhized plants (Control) without *N. aberrans*
Non-mycorrhized plants (Control) with *N. aberrans*
Mycorrhized plants with *Funneliformis moseeae,* without *N. aberrans*
Mycorrhized plants with *Funneliformis moseeae* with *N. aberrans*
Mycorrhized plants with *Rhizoglomus intraradices* A2 without *N. aberrans*
Mycorrhized plants with *Rhizoglomus intraradices* A2 with *N. aberrans*
Mycorrhized plants with *Rhizoglomus intraradices* B1 without *N. aberrans*
Mycorrhized plants with *Rhizoglomus intraradices* B1 with *N. aberrans*

Inoculation was carried out by making three holes in the substrate around the plant, depositing 1.5 mL of inoculum in each hole and then covering the holes with the same substrate.

Experimental design

The test had a completely randomized experimental design and the treatments a factorial arrangement, being two factors: mycorrhization and infestation, with 4 and 2 levels, respectively. Each of the 8 treatments consisted of 8 plants, each of them constituting a repetition.

Statistical analysis

The data were analyzed by ANOVA, and the mean comparison test was performed by Fisher's LSD test using the SISVAR program. For the statistical analysis of variables whose results were expressed in percentage, these were previously transformed by the arcsine function.

Analyzed variables

At 90 days after the transplant, non-destructive physiological determinations were made: Net photosynthesis (IRGA: CIRAS-2® model, PP Systems), stomatal conductance, and stem diameter measurements.

At 100 days, the plants were removed and the leaves, root, and soil samples were taken. Material was collected to make the following determinations:

- Fresh root and dry aerial weight
- Total chlorophyll (Wellburn 1994). A leaf disc 1 cm in diameter was placed in 1.5 mL of N–Dimethylformamide. The absorbances of the solution were measured at wavelengths 646.8, 663.8, and 480 nm, in a Shimadzu UV160 A spectrophotometer.

  $\text{Chlorophyll } (\mu g\ cm^{-2}) = 12 \times Ab\ 663.8 - 3.11 \times Ab\ 646.$
  $\text{Chlorophyll b } (\mu g\ cm^{-2}) = 20.78 \times Ab\ 646.8 - 4.88 \times Ab\ 663.8$
  $\text{Total Chlorophyll a+b } (\mu g\ cm^{-2}) = 17.67 \times Ab\ 646.8 + 7.12 \times Ab\ 663.8$

- Soluble proteins in leaf and root tissue (Bradford 1976). Two hundred milligrams of tissue was homogeneized with a 1 mL extraction buffer (TRIS 50 mM, EDTA 1 mM, PVPP insoluble 1%, MeSH 0.1% a pH 7.5) at 4 °C. The resulting homogenate was centrifuged at 10,000 g for 10 min at 4 °C. One hundred microliter of the supernatant was taken and 5 mL of Coomassie's Bright Blue reagent was added, stirred in vortex, and the absorbance was read at 595 nm. The calculation of the protein concentration was carried out using a standard curve prepared with different concentrations of bovine serum albumin (BSA, Sigma Chemical Co).
- Number of eggs housed in the roots. The entire root was placed in a blender and the agitation extraction technique was applied. (Hussey and Barker 1973; Coolen 1979) with subsequent purification and concentration by the centrifugation–flotation technique (Coolen 1979).
- Number of mobile forms in soil (Coolen 1979). One hundred microliters of pot substrate was placed and 5 L of water was added. It was shaken and the purification and concentration by the centrifugation–flotation technique (Coolen 1979) was applied. The number of vermiforms counted was multiplied by the total volume of the pot.
- Final population of the nematode: Eggs housed in the root + Vermiforms in soil
- Reproduction factor: Final Population/Initial population (Oostenbrink 1966).
- Malonyldialdehyde (MDA) content in leaf and root tissue (Heath and Packer 1968). Two hundred micrograms of tissue was macerated with 1 mL of 0.1% trichloroacetic acid and centrifuged for 10 min at 10,000 g. The supernatant was reacted with 1 mL of the reagent TCA–BHT–TBA (TCA 20%, thiobarbituric acid (TBA) 0.37% and butylhydroxytoluene (BHT) 0.01 g) and the tubes were incubated for 30 min at 95 °C. After this period, they were placed in an ice bath to stop the reaction and centrifuged at 3000 g for 10 min. Finally, the supernatant was separated and the absorbance was read at 532 and 600 nm on a Shimadzu UV 160 UV/V spectrophotometer. The MDA concentration was calculated using an extinction coefficient of 155 mM cm^{-1}: MDA equivalents (nmol ml^{-1}) = [(A532 – A600) / 155000].
- Proline content in leaf and root tissue (Bates et al. 1973). One hundred milligrams of fresh tissue was homogenized with 2 mL of sulfosalicylic acid 3%. It was centrifuged at 12,000 g for 15 min and 1 mL of the supernatant was taken. It was reacted with 1 mL of the acid reagent Ninhydrine and 1 mL of glacial acetic acid in a 15-mL tube, in a water bath at 100 °C for 1 h. The reaction was then interrupted by cooling the samples in ice. To the previous mixture, 2 mL of toluene was added and stirred for 15 s in vortex. The phases were allowed to separate, and the aqueous phase containing the toluene–proline chromophore was taken. Absorbance was measured at 520 nm using toluene as a target. Proline content was calculated according to µmol proline.g^{-1}FW = [(µg proline/mL × mL toluene)/115.5 µg/µmoles]/[(g FW)/5
- Total phenol content in leaf and root tissue (Singleton and Rossi 1965). Five hundred milligrams of fresh tissue was homogenized in ethanol. The mixture was centrifuged at 10,000 g for 15 min at 4 °C. Forty microliters are extracted from the sample and mixed with distilled

water, and the Folin–Ciocalteu reagent 1 N. After 3 min at 25 °C, the Na2CO3 2% (w/v) solution was added in NaOH 0.1 N. It was incubated for 90 min at 25 °C, and the absorbance was measured at 760 nm in a spectrophotometer. A standard curve was made based on different concentrations of gallic acid.

- Relative conductivity of leaf and root tissue (Lutts et al. 1996). Two hundred milligrams of tissue was weighed and washed three times with doubly distilled water for 15 s. Each sample was immersed in a tube with 10 mL of doubly distilled water where they remained for 4 h at room temperature. Subsequently, the electrical conductivity (dS m − 1) was determined using a Jenco conductivity meter model 3173. The tubes were then plugged and autoclaved (20 min, 120 °C, 1 atmosphere pressure). Finally, the tubes were allowed to cool to room temperature and the electrical conductivity was measured again. The relative conductivity of cell membranes was estimated from the following formula: CR (%) = (L1 / L2) × 100, where L1 and L2 are the electrical conductivity readings before and after autoclaving, respectively.
- Percent of mycorrhizal colonization (Phillips and Hayman 1970). Root fragments were immersed in KOH 10% (w/v), 10 min at 100 °C. They were then washed three times with distilled water and a 0. 1N HCl solution was applied, 5 min at 20 °C. Structure staining was performed with trypan blue, 5 min at 95 °C. The roots are placed on slides, lactic acid drops are added, and observed under an optical microscope.
- Viability of fungal structures (Smith and Gianinazzi-Pearson 1990). Root fragments were placed for 18 h in a 0.2 M Tris–HCL solution, pH 7; 2.5 M sodium succinate.$6H_2O$; 5 mM $MgCl_2$ and 4 mg/mL nitroblue tetrazolium. After that time, they were rinsed with a 75% chloral hydrate solution. Finally, they were placed in a 0.01% acid-fuchsin solution in lactic acid for 8 min at 90 °C. The root fragments are observed under the microscope, and the percent of viable and active fungal structures that are colored blue is quantified.

Results and discussion

Parameters linked to AMF

The root staining and the quantification of the percent of mycorrhizal colonization performed prior to transplantation, confirmed that the roots were colonized by the three isolates used. The percent of mycorrhization was 85%, 76%, and 72% for Fm, Ri–B1, and Ri–A2, respectively.

Staining of root samples at the end of the test indicated that parasitism of *N. aberrans* caused an average reduction of 19 points in the percent of colonization in Ri–A2. In turn, comparing the different AMF in the presence of the nematode, Ri–A2 was the isolate that presented the least mycorrhization (Table 1). The other isolated showed no statistical differences when the nematode was present.

Regarding the viability of the fungal structures Ri–A2 and Fm maintained values without significant differences in the presence and absence of the nematode, while Ri–B1 had a reduction of 17.8 points when the nematode was present. In the parasitized treatments, Ri–B1 was the isolate that presented significantly lower values of fungal viability (Table 1).

Most research involving AMF and plant parasitic nematodes focuses on parasite control, without studying in detail what are the effects on fungi. The results presented here show that the isolates of the genus *Rhizoglomus* used in this assay have some type of interaction with the nematode since it impacts the colonization and/or the viability of the fungal structures. Jung et al. (2012) and Vos et al. (2014) suggest that photoassimilates are a scarce resource and there is competition for them between both populations, which affects the optimal development of both. The syncytia of *N. aberrans* and the arbuscules of the AMF develop in the root pericycle, so it is possible that there is a competition for physical space in addition to what is hypothesized with respect to photoassimilates. These considerations suggest that early inoculation with AMF at the planting and growth stage of the seedling in the tray will have a positive impact on AMF/nematode competition. Mycorrhizal symbiosis will be established before transplanting, at which time the root will come into contact with the nematodes present in the soil. Mycorrhizal symbiosis is a dynamic interaction between the fungus and the plant that undergoes alterations due to the influence of various factors. In a test where isolates are

Table 1 a) Percent of mycorrhizal colonization and b) viability of the fungal structures of three arbuscular mycorrhizal fungi in tomato lants grown in the presence and absence of *Nacobbus aberrans*

Treat.	Without *N. aberrans*	With *N. aberrans*
a)		
Control	0 ± 0 Ab	0 ± Ac
Ri–A2	49.79 ± 5.9 Aa	30.27 ± 9.1 Bb
Ri–B1	45.63 ± 9.2 Aa	55.78 ± 8.8 Aa
Fm	62.95 ± 14.3 Aa	46.71 ± 5.4 Aa
b)		
Control	0 ± 0 Ac	0 ± 0 Ac
Ri–A2	31.18 ± 8.2 Aa	37.8 ± 12.6 Aa
Ri–B1	40.16 ± 7.7 Aab	22.3 ± 10.02 Bb
Fm	43.16 ± 6.7 Ab	43.7 ± 5.73 Aa

Capital letters on the line compare the same mycorrhizal condition in the absence and presence of the nematode. Lowercase letters in the circle compare the different AMF under the same nematological condition. Means accompanied by different letters indicates significant differences according to Fisher's LSD test ($p \leq 0.05$)

compared, the viability test is of paramount importance, since the Tripan blue staining technique does not differentiate between active and non-active structures. In tests where co–inoculations of mycorrhizae and galling nematodes have been carried out, it is observed that not all isolates are affected in the same way and even some seem to be stimulated by the presence of the nematode (Pinochet et al. 1998; Cofcewicz et al. 2001).

Parameters linked to the population of *N. aberrans*

The quantification of eggs lodged in the roots indicated that mycorrhization caused a significant reduction in them, with no significant differences between the 3 isolates of AMF (Fig. 1a).

In the count of mobile forms extracted from 100 mL of soil, non-mycorrhizal treatment presented the highest number of individuals (Fig. 1b). Mycorrhization resulted in reductions in the Ri–B1 and Fm treatments compared to the uninoculated control.

Accounting for the final population values of nematodes recorded, reductions of 71%, 51%, and 73% were measured for the Ri–A2, Ri–B1, and Fm treatments, respectively (Fig. 1c).

The results allow us to conclude that the symbiosis between the tomato and any of the 3 isolates of AMF had an antagonistic effect over the *N. aberrans* population. For this essay, we can rule out at least one of the explanations suggested by Barea et al. (2002) which include the possibility that the protective effect comes from the development of antagonistic microorganisms growing stimulated by hyphae in the rhizosphere, since the substrate used in the test was previously sterilized by tyndallization.

Among the possible justifications for why mycorrhizal plants cause a decrease in the population of phytoparasitic nematodes, the following can be considered:

An improvement in the nutritional status of the host plant. The association of mycorrhizae allows a greater absorption of nutrients such as P, N, K, Ca, Mg, Fe, and Mn resulting in more vigorous and more pathogen–tolerant plants (Gianinazzi et al. 2010).

Changes in the root system: after colonization, the mycorrhizal fungus causes morphological and physiological changes in the root, which hinder the entry of other microorganisms, or in the case of endoparasitic nematodes, syncytia of smaller numbers of cells are formed (Fassuliotis 1970; Pozo et al. 2013).

Modifications in root exudates: The establishment of symbiosis generates qualitative and quantitative changes in root exudates. This results in the appearance of substances that exert a direct effect on harmful microorganisms or alter the plant/pathogen signaling, decreasing the entry of juveniles into the root (Sikora and Fernández 2005).

Synthesis of compounds: Mycorrhiza can stimulate the production of substances involved in the plant's defense system, such as phytoalexins, phenolic compounds, enzymes (peroxidases, beta–glucanases, ammonioliases phenylalanine, chitinases, polyphenol oxidases) and other proteins (Solórzano et al. 2001; Vos et al. 2012).

Classical biological control: some AMF behaves in certain circumstances as weak parasites of the eggs of some nematodes (Francl and Dropkin 1985; Tribe 1977), and this phenomenon could be contributing to some extent to explain the mechanism of inhibition.

Parameters linked to plant growth and physiology

Prior to the completion of the test, with the plants still standing, we proceeded to measure the stomatal conductance (CEs) and net photosynthesis (PN). The analysis of the variance of the CEs did not show a significant interaction between the factors mycorrhization and infestation

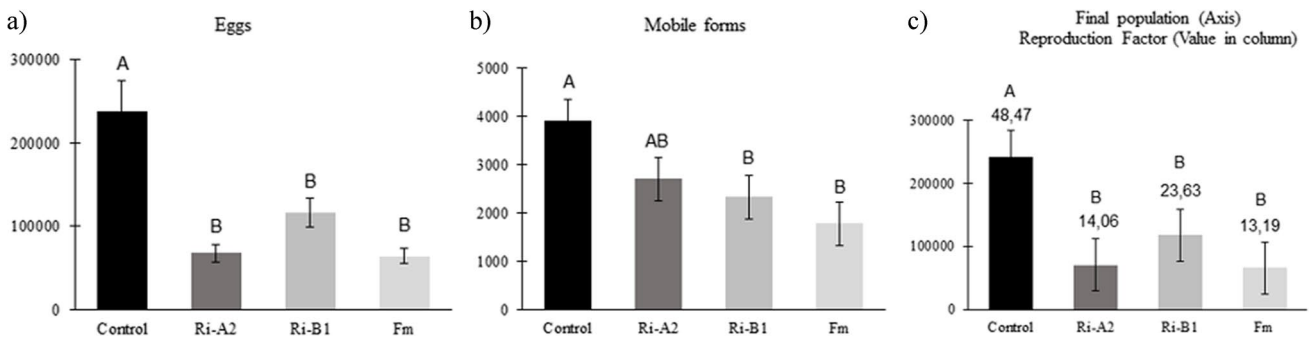

Fig. 1 Eggs in roots, number of mobile forms extracted from the soil, final population, and reproduction factor of Platense tomato plants inoculated with three arbuscular mycorrhizal fungi. Ri–A2, *Rhizoglomus intraradices* A2; Ri–B1, *Rhizoglomus intraradices* B1; Fm, *Funneliformis moseeae*. Stockings accompanied by different letters indicate significant differences according to Fisher's LSD test ($p \leq 0.05$)

with the nematode ($p = 0.32$); however, there were significant differences between the levels of these main factors. The gas exchange of plants inoculated with Ri–B1 and Fm was greater than that of control plants and those inoculated with Ri–A2. Plants parasitized by *N. aberrans* had a lower stomatal conductance than non-parasitized plants (Table 2).

In the absence of the nematode, the lowest PN was recorded in Ri–A2. Comparing parasitized and non-parasitized plants, control plants had a significant reduction in CO2 fixation; Ri–A2 and Ri–B1 showed no changes and, although Fm had a reduction, maintained values significantly higher than the non-mycorrhized control. In the presence of *N. aberrans*, Ri–A2 had a similar PN to that of the control treatment (Fig. 2).

Measurements linked to plant growth, such as dry mass of the aerial part and stem diameter in the middle part of the plants, showed no significant differences between treatments. In the case of fresh root weight in the absence of the nematode, the mycorrhizal plants with Fm had a lower weight than the rest of the treatments, but in the presence of the nematode, the weight remained unaffected. In the control plants, the attack of the nematode reduced the fresh root weight (Table 3).

The concentration of chlorophyll in the leaves in the absence of the nematode was lower in the Ri–A2 treatment. Comparing parasitized and non-parasitized plants, control plants and Fm had a reduction in chlorophyll content as a result of parasitism, while Ri–A2 and Ri–B1 plants did not present significant differences (Table 3).

In the absence of the nematode, no advantages were observed in the growth and physiological processes of mycorrhizal plants over non-mycorrhizal plants. The fresh root weight of the three mycorrhizal treatments was lower than that of the controls. It is known that the proliferation of hyphae fulfilling absorption functions made plants allocate fewer resources to root growth (Pereira et al. 2001).

In the presence of the nematode, a reduction in PN, total chlorophyll and fresh root weight are observed for control plants; shrinkage that does not occur in mycorrhizal plants. Comparing the 3 isolates of mycorrhiza, RI–A2 presented PN values and total chlorophyll significantly lower than RI–B1 and Fm.

Table 2 Stomatal conductance of Platense tomato plants: a) inoculated with different arbuscular mycorrhizal fungi, b) inoculated and not inoculated with *Nacobbus aberrans*

Treatment	Mean
a)	
Control	90.4 ± 17.9 b
Ri–A2	92.3 ± 16.2 b
Fm	113.2 ± 13.2 a
Ri–B1	120.9 ± 29.2 a
b)	
Without *N. aberrans*	116.0 ± 23.8 a
With *N. aberrans*	92.4 ± 16.6 b

Means accompanied by different letters, indicates significant differences according to Fisher's LSD test ($p \leq 0.05$)

Ri–A2 *Rhizoglomus intraradices* A2, Ri–B1 *Rhizoglomus intraradices* B1, Fm *Funneliformis moseae*

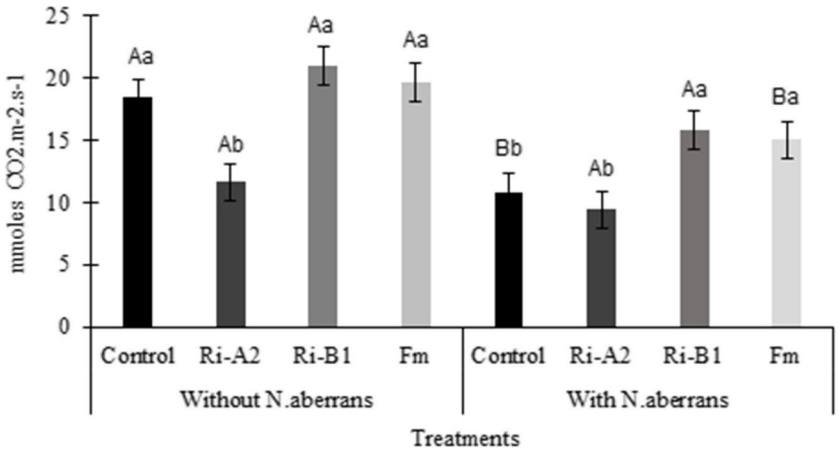

Fig. 2 Net photosynthesis in mycorrhizal tomato plants with 3 arbuscular mycorrhizal fungi in the presence or absence of *Nacobbus aberrans*. Ri–A2, *Rhizoglomus intraradices* A2; Ri–B1, *Rhizoglomus intraradices* B1; Fm, *Funneliformis mosseae*. Capital letters compare the same mycorrhizal condition in the absence and presence of nematodes. Lowercase letters compare different mycorrhizae within the same nematological condition. Means accompanied by different letters indicate significant differences according to Fisher's LSD test ($p \leq 0.05$)

Table 3 Parameters linked to the growth of tomato plants inoculated with 3 mycorrhizal forming fungi in the presence or absence of *Nacobbus aberrans*

	Without *Nacobbus aberrans*				With *Nacobbus aberrans*			
	Control	Ri–A2	Ri–B1	Fm	Control	Ri–A2	Ri–B1	Fm
Fresh root weight (g)	163.7 Aa	132.2 Aab	135.2 Aab	111 Ab	130.7 Ba	108.2 Aa	143.8 Aa	124.7 Aa
Aerial dry weight (g)	60.04 Aa	58.18 Aa	62.02 Aa	57.16 Aa	60.58 Aa	61.3 Aa	62.0 Aa	58.44 Aa
Total chlorophyll (µg.cm^{-2})	10.28 Aa	7.23 Ab	9.93 Aa	11.9 Aa	7.3 Bb	7.42 Ab	10.1 Aa	10.2 Ba
Stem diameter (cm)	1.2 Aa	1.2 A a	1.16 Aa	1.21 Aa	1.06 Aa	1.1 Aa	1.19 Aa	1.1 Aa

Capital letters compare the same mycorrhizal condition in the absence and presence of the nematode. Lowercase letters compare different mycorrhizae within the same nematological condition. Means accompanied by different letters indicate significant differences according to Fisher's LSD test ($p \leq 0.05$)

Ri–A2 *Rhizoglomus intraradices* A2, Ri–B1 *Rhizoglomus intraradices* B1, Fm *Funneliformis mosseae*

Under controlled conditions, in the absence of stressors, mycorrhization may not present quantifiable productive benefits. The mycorrhizal fungus can become an important destination for photoassimilates that cause a reduction in growth in the plant. On the other hand, the extensive growth of hyphae in the substrate that is one of the main benefits that the fungus gives to the plant could be limited because it is a pot test.

Mycorrhizal symbiosis, in addition to making significant contributions to the host, fulfills important ecological functions. The carbon supplied by the plant to the AMF is not exclusively for consumption, but through the hyphae, a flow of carbon from the atmosphere to the soil is established that causes significant increases in biological activity (Finlay and Söderström 1992). Not all isolates manifest the same efficiency to perform this task. It could be expected that for cases where what is sought is to remedy a degraded or contaminated soil, this type of isolate would be the most beneficial, but not in jobs where the priority is placed on the productivity of the host.

The content of soluble proteins in the leaf tissue of mycorrhizal plants ranged from 19.9 to 28.03 µg of protein.mg^{-1}, both in the absence and in the presence of the nematode, without significant differences between the different isolates. The control treatment plants + *N. aberrans* had a significantly lower protein content than the controls without nematode and lower than the mycorrhized with nematode (Fig. 3a).

The soluble proteins of the root tissue presented a different behavior. In the absence of the nematode, mycorrhizal plants had a lower concentration of proteins compared to the control treatment. In the presence of the nematode, mycorrhizal plants with Ri–B1 and Fm had a higher protein content than control plants and mycorrhizal plants with Ri–A2. Comparing parasitized plants with non-parasitized plants, it was verified that in mycorrhizal roots, the protein content

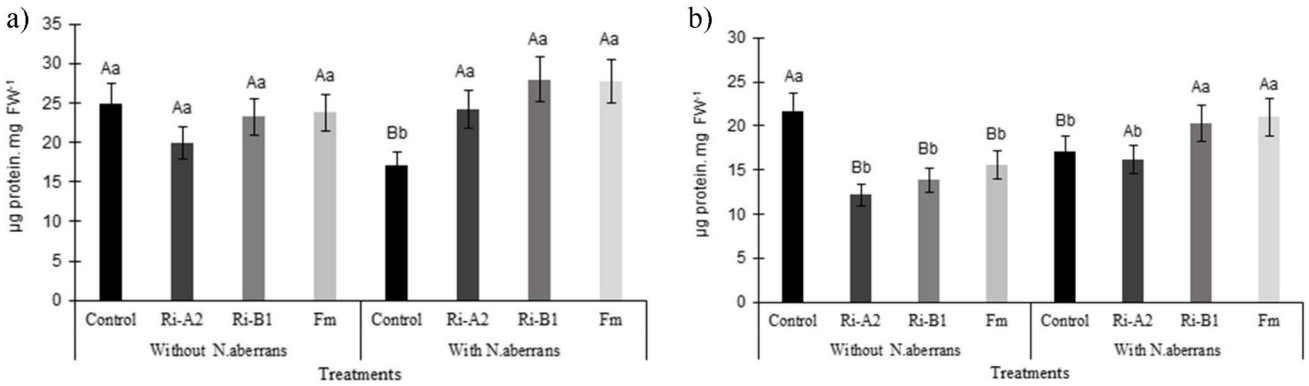

Fig. 3 Soluble protein content in **a** leaf tissue and **b** root tissue of mycorrhized Platense tomato plants with 3 arbuscular mycorrhizal fungi in the presence or absence of *Nacobbus aberrans*. Ri–A2, *Rhizoglomus intraradices* A2; Ri–B1, *Rhizoglomus intraradices* B1; Fm, *Funneliformis moseae*. Capital letters compare the same mycorrhizal condition in the absence and presence of nematode. Lowercase letters compare different mycorrhizae within the same nematological condition. Columns accompanied by different letters indicate significant differences in the Fisher LSD test ($p \leq 0.05$)

was increased by the presence of the nematode, while in non-mycorrhizal roots, it was reduced (Fig. 3b).

The protein content in the aerial parts reflects what has been observed in other growth parameters. Control plants are affected by the nematode and mycorrhized plants to a greater or lesser extent manage to mitigate the negative impact of parasitism. In the case of the root, the results are not so clear and other types of studies should be carried out to know how the protein pattern is modified in each case. The modifications that occur in the root by the presence of a symbiont require important changes in the plasma and vacuolar membranes that alter the expression of genes that encode proteins (Krajinski et al. 2000). It has also been well documented that nematode attack stimulates the synthesis of defense proteins (Mejias et al. 2019). It remains to be studied in greater depth how the protein metabolism of the root is modified in the presence of a symbiont and a pathogen.

The quantification of phenols in leaf tissue showed no differences between treatments. In all cases, the concentration was between 1.35 and 1.40 µg mg of fresh tissue weight.

Table 4 Total phenol content expressed in µg mg fresh weight of root tissue of mycorrhized tomato plants with 3 arbuscular mycorrhizal fungi in the presence and absence of *Nacobbus aberrans*

Treatments	Without *N. aberrans*	With *N. aberrans*
Control	3.41 ± 1.3 Bc	5.42 ± 0.68 Aa
Ri–A2	5.74 ± 0.46 Aa	4.90 ± 1.2 Aa
Ri–B1	5.27 ± 0.58 Aab	4.78 ± 0.28 Aa
Fm	4.75 ± 0.37 Ab	5.27 ± 0.34 Aa

Capital letters on the line compare the same mycorrhizal condition in the absence and presence of the nematode. Lowercase letters in the column compare the different AMF under the same nematological condition. Means accompanied by different letters indicate significant differences according to Fisher's LSD test ($p \leq 0.05$)

In the root tissue, the concentration of this metabolite was higher than that quantified in the leaves. In the absence of *N. aberrans*, the phenol content of mycorrhized roots was significantly higher than that of non-mycorrhized roots. In the presence of the nematode, the phenol content of the mycorrhized roots remained unchanged, while in the control plants there was a significant increase (Table 4).

The concentration of proline in leaves was increased in all parasitized treatments compared to non-parasitized treatments. The Ri–A2 treatment, in the presence of the nematode, showed the highest amount of proline compared to the other treatments inoculated with AMF (Fig. 4a).

The concentration of proline in roots was increased in control, RI–A2 and RI–B1 parasitized treatments compared to non-parasitized treatments, but not in those inoculated with FM where the presence of the nematode did not cause changes in proline levels. Control and Fm treatments had the highest levels of proline in the absence of the nematode. In the presence of the nematode, all mycorrhizal treatments had lower proline levels than control (Fig. 4b).

The mallonyldialdehyde (MDA) content and relative tissue conductivity (CR) of the leaves were not modified by nematode parasitism. Both in the absence and in the presence of the nematode, the Ri–A2 leaves presented higher MDA values than the control (Table 5).

In root tissue, *N. aberrans* caused an increase in MDA content and %CR in non-mycorrhizal plants. Not so in mycorrhized plants, whose values were not altered, indicating a better condition of cell membranes.

The parasitism of *N. aberrans* causes mechanical damage to the membranes and disorders in the absorption of water and nutrients that mainly trigger water and nutritional stress. In this section, it is observed how mycorrhizal symbiosis acts attenuating at least partially, such damages.

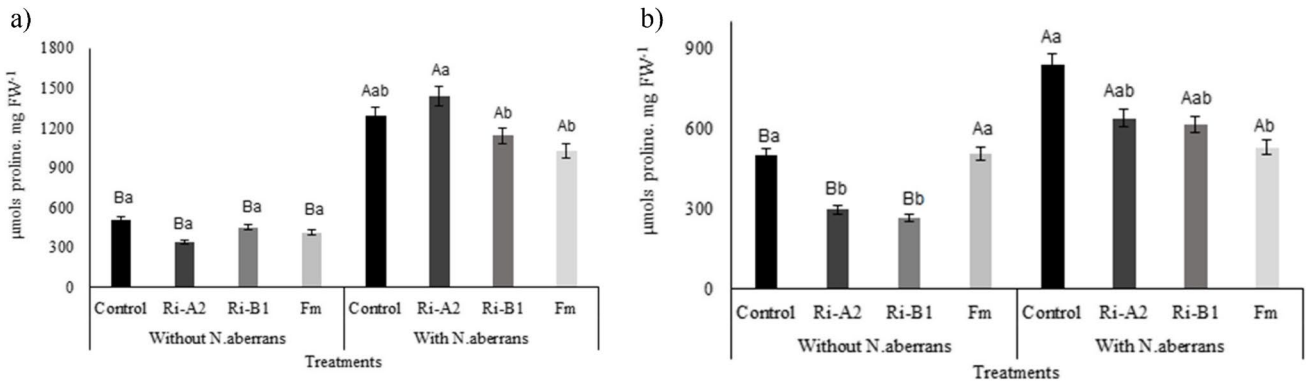

Fig. 4 Proline concentration in **a** leaf tissue **b** root tissue of mycorrhizal tomato plants with 3 arbuscular mycorrhizal fungi in the presence and absence of *Nacobbus aberrans*. Capital letters compare the same mycorrhizal condition in the absence and presence of the nematode. Lowercase letters compare different mycorrhizae within the same nematological condition. Columns accompanied by different letters indicate significant differences according to Fisher's LSD test ($p \leq 0.05$)

Table 5 Content of mallonyldialdehyde and relative tissue conductivity, foliar, and root of mycorrhized tomato plants with 3 arbuscular mycorrhizal fungi in the presence and absence of *Nacobbus aberrans*

	CV	Without *Nacobbus aberrans*				With *Nacobbus aberrans*			
		Control	Ri–A2	Ri–B1	Fm	Control	Ri–A2	Ri–B1	Fm
Foliar MDA (nmoles.gr FW^{-1})	22%	7.75 Ab	11.94 Aa	10.4 Aab	8.46 Ab	7.24 Ab	11.4 Aa	9.26 Aab	9.45 Aab
Radicular MDA (nmoles.gr FW^{-1})	15.1%	2.01 Ba	2.36 Aa	2.20 Aa	2.22 Aa	3.05 Aa	2.31 Ab	2.06 Ab	2.21 Ab
Foliar CR (%)	23%	16.98 Aa	16.22 Aa	19.57 Aa	18.10 Aa	19.14 Aa	14.85 Aa	19.14 Aa	21.19 Aa
Radicular CR (%)	26.3%	23.77 Ba	20.35 Aa	22.88 Aa	23.71 Aa	32.01 Aa	25.33 Aab	27.49 Aab	23.22 Ab

Capital letters on the line compare the same mycorrhizal condition in the absence and presence of the nematode. Lowercase letters in the column compare the different AMF under the same nematological condition. Different letters indicate significant differences according to Fisher's LSD mean comparison test ($p \leq 0.05$)

In this work, mycorrhization caused a significant increase in the content of phenolic compounds of root tissue, a fact that was also documented by other authors (Soares et al. 2005). The functionality of phenolic compounds in plant defense is broad and often difficult to determine. Some compounds such as chlorogenic acid and hydroxycinnamic acids can be, in themselves, toxic to pathogens; other can rapidly develop into reactive oxygen species that act in response to attack (Hartleb et al. 1997). The higher phenol content of mycorrhized roots in the absence of the nematode suggests some kind of resistance induction or priming. This cannot be affirmed, since in order to do so, it is necessary to verify that some metabolic pathway linked to plant defense has been stimulated or that some group of genes is being overexpressed (Madriz Ordeñana 2002).

Proline is a metabolite that accumulates in plant tissues in response to water deficits. Its accumulation decreases the osmotic potential of the cells, favoring the absorption and movement of water in the plant. Proline, in addition to acting as an osmoprotective, plays an important role in blocking reactive oxygen species (Hossain et al. 2014).

The exploration of the substrate by the hyphae facilitating the absorption of water explains the lower levels of proline in situations where, as a result of parasitism, the functionality of the root is affected. In addition, some authors indicate that mycorrhization stimulates, at the root, the expression of genes that encode aquaporins (membrane proteins involved in water absorption) (Ruiz Sánchez et al 2011).

Malondialdehyde is the main product of the peroxidation of cell membrane lipids by the action of reactive oxygen species (Bhattacharjee et al. 2014). The measurement of the relative electrical conductivity of tissues reflects the ability of membranes to retain electrolytes (Cseresnyés et al. 2016). These indicators allow the evaluation of oxidative stress and damage to plasma membranes. The less damage to the membranes of mycorrhizal plants may be due to a lower attack by the nematode in the plants or to a higher antioxidant activity. Several authors agree that mycorrhization stimulates the activity of some enzymes such as superoxide dismutase, peroxidase, and catalase (Lambais et al. 2003). Borde et al. (2011) also suggests that symbiosis stimulates the production of antioxidant enzymes that allow plants a greater ability to respond to and neutralize reactive oxygen molecules.

Conclusions

Mycorrhizal association is beneficial in conditions where "inverted" photoassimilates are used to reverse or compensate for a stressful situation. The crops, unlike the conditions given in this test, are continuously exposed to various adverse situations of different magnitude. The possibility of mycorrhiza becoming a symbiont that does not offset "the costs with its benefits is unlikely."

When *N. aberrans* and AMF are living together in the same host, there is an interaction between them, either directly or indirectly, that causes modifications in their respective populations, as well as in plant growth and physiology. The three isolates used here managed to reduce the population of *N. aberrans* in tomato plants. However, in some parameters such as stomatal conductance, net photosynthesis, and chlorophyll content RI–A2 showed inferior performance than the other isolates. The evaluation of the colonization of the fungus in the root and the survival of its structures shows that *Funneliformis mosseae* had a better performance. This last characteristic is important if the use of this isolate as a bioinput for the integrated management of nematodes is planned.

According to the results obtained, it is recommended that inoculation with AMF be carried out at planting so that the symbiosis is established early before the plants are exposed to stress situations. The inoculum of AMF that was elaborated for the realization of this work does not present difficulties for its management, conservation, and application in plants, promising the use of this biotechnology in productive regions affected by *N. aberrans*.

Author contribution All authors contributed to the study conception and design. Material preparation, data collection, and analysis were also

performed by all authors. The first draft of the manuscript was written by the first author, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Data availability The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Competing interests The authors declare no competing interests.

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