

Solanum melongena ecophysiology under the influence of *Meloidogyne javanica*

ABSTRACT

Aim: The aim of this study was to quantify the ecophysiological changes in eggplant cv. Embu (*Solanum melongena* L.) conducted in the presence and absence of *Meloidogyne javanica*.

Study design: The experimental design has completely randomized with two groups of plants (with and without soil infestation) with five replications and two plants per plot.

Place and Duration of Study: The experiment has been carried out at the Agrarian Sciences Center of the Federal University of Paraíba, Areia, PB, between October and December 2017.

Methodology: Used eggplant cultivar was the Embu, the seedlings have been transplanted to 5 dm³ pots, filled with a substrate formulated by the mixture of vegetal soil, sand and bovine manure in the proportion of 3:1:1. Growth characteristics have been evaluated at 60 days after transplanting (DAT), and the following variables were measured: plant height, stem diameter, leaves number, flowers number, aerial, root and total dry mass and Dickson quality index. Gas exchange evaluations have been performed at the period from 7:00 am to 5:00 pm. Chlorophyll fluorescence emission measurements have been performed.

Results: For variables growth, flowering and chlorophyll there was no significant difference between plants with and without inoculation of *M. javanica*, evidencing that they were resistant to the population level to which they had been submitted. In plants inoculated with *M. javanica* have been observed a bigger increase in the rate of CO₂ assimilation and in the carboxylation efficiency.

Conclusion: Therefore, it has affirmed that these plants have mechanisms of regulation of their photosynthetic activities, which gives them tolerance to the stress of *M. javanica*, causing no damage to their growth.

Keywords: Knot nematodes; gas exchange; physiological behavior.

1. INTRODUCTION

Eggplant (*Solanum melongena* L.) is a species belonging to the Solanaceae botanical family, originating from countries of tropical eastern regions, such as China and India, presenting high economic importance due to its nutraceutical properties [1]. It is notable among consumers for the increase in demand for this oleracea, one of the possible explanations for this increase is that in addition to consumers seeking healthier products, eggplant has proven nutritional and medicinal properties [2]. In addition, eggplant is a plant which contains lowering cholesterol compounds and is widely used as an alternative treatment for plasma cholesterol [3,4].

In general, oleraceas have been commonly affected by diseases caused by phytopathogenic microorganisms [5]. Even though, it is considered a rustic species, the eggplant shows sensitivity to the attack of pathogens, which without prevention or even adequate control can cause damages and alter the quality of the final product [6].

The nematodes of the genus *Meloidogyne* have as main characteristic the formation of knots in the root system of the cultures, in the eggplant culture, in particular, they have already become one of the most important pathogens, since they are difficult to control [7]. When conducted under high population densities the photosynthetic rate, growth and crop yields tend to reduce [8].

Faced with such facts, there is little information on the physiological activity of olerocarpa plants, especially eggplant, under the influence of soil pathogens, justifying this research. There is also little information on the behavior of plants in different phenological stages when exposed to population densities of knots nematodes.

This information may be useful for the genetic improvement of the culture regarding the attack of these pathogens, since they are difficult to control. Thus, the aim of this research was to quantify the ecophysiological changes in eggplant (*Solanum melongena* L.) plants conducted in the presence and absence of *Meloidogyne javanica*.

2. MATERIAL AND METHODS

The research has been carried out in a greenhouse belonging to the Department of Plant Science and Environmental Sciences of Federal University of Paraíba, UFPB, located in the municipal district of Areia, Paraíba, Brazil.

The experimental design has been completely randomized with two groups of plants (with and without soil infestation) with five replications and two plants per plot. In order to obtain the inoculum, a population of *Meloidogyne javanica* was maintained and multiplied in tomato plants (*Solanum lycopersicum* L. cv. Santa Clara) which were cultivated in pots with 2 dm³ of soil of ravine and sand (2:1) and maintained in a greenhouse during the period of 70 days.

The nematode extraction has been performed according to the methodology suggested by Hussey and Barker [9] and adapted by Boneti and Ferraz [9]. The aerial part of the tomato plants has been separated from the root system and the roots washed, sectioned and ground in a blender in 0.5% sodium hypochlorite (NaClO) solution, under low rotation for 20 seconds. The solution has been passed in 200 and 500 mesh sieves (74 and 26 mm apertures, respectively). Contents of 500 sieve have been washed with water to remove all sodium hypochlorite and then collected in beaker and quantified under an optical microscope. Then, it has been carried out a soil infestation of a group of plants with 6,000 eggs of *Meloidogyne javanica* per pot.

Used eggplant cultivar was the Embu (Feltrin®). The seedlings have been produced in polyethylene trays using commercial substrate (Basaplant®). Three seeds have been sown per cell, and the excess seedlings have been removed after emergence. Transplanting has been carried out when plants reached 10-15 cm height, and the seedlings have been transplanted to 5 dm³ pots, filled with a substrate formulated by the mixture of vegetal soil, sand and bovine manure in the proportion of 3:1:1, pre-sterilized in an autoclave at a temperature of 120 °C, 1 atm of vapor pressure for 2 hours. Irrigations have been performed whenever there was a need to maintain soil moisture close to the field capacity.

Table 1. Chemical characteristics of substrate components used in the experiment.

pH	P	K ⁺	Na ⁺	H ⁺ +Al ⁺³	Al ⁺³	Ca ⁺²	Mg ⁺²	SB	CEC	OM
	--- mg kg ⁻³ ---			----- cmol _c dm ⁻³ -----						g kg ⁻¹
6.1	79.68	252.02	0.11	2.62	0.0	3.32	1.98	6.06	8.68	23.58

SB = Sum of bases; CEC = Cation exchange capacity; OM = Organic matter

Growth characteristics have been evaluated at 60 days after transplanting (DAT), and the following variables were measured: plant height, using ruler graduated in centimeters, measuring from plant collar to the apical bud; stem diameter, carried out with the aid of a digital caliper, and results being expressed in mm; number of leaves has been made from the leaf count, considering photosynthetically active leaves; flower buds has been established by counting the buds found in the plant; the aerial, root and total dry mass has been measured at the end of the experiment when the aerial and radicular parts have been separated at the height of the plant neck, packed in Kraft paper bags and put to dry in forced air circulation until they reach constant weight. Subsequently, the analytical scale has been weighed with an accuracy of 0.001g. The total dry mass was determined from the sum of the dry masses of the aerial part and the root. The results have been expressed in g plant⁻¹; Dickson quality index has been established by the relationship between plant height (H), stem diameter (SD), dry shoot mass and dry root mass, according to Dickson et al. [10].

$$DQI = \frac{TDM}{H \text{ (cm)}/SD \text{ (mm)} + SDM \text{ (g)} / RDM \text{ (g)}}$$

Where: TDM = total dry mass; H = height; SD = stem diameter; SDM = shoot dry mass; DMR = root dry mass.

Physiological evaluations have been performed on the third leaf from the apex, at the period from 7:00 am to 5:00 pm, with intervals of 2 hours, totaling 6 evaluations. The infrared gas analyzer (IRGA, LI-COR® 6400-XT, Lincoln, USA) was used to analyze the rate of assimilation of CO₂ (A) (μmol CO₂ m⁻² s⁻¹), transpiration (mmol H₂O m⁻² s⁻¹), internal CO₂ concentration (Ci) (μmol CO₂ mol⁻¹), stomatal conductance (gs) (mmol H₂O m⁻² s⁻¹) and leaf temperature (°C). Water use efficiency (WUE - A/E), intrinsic water use efficiency (iWUE - A/gs) and instantaneous carboxylation efficiency (iCE - A/Ci) have quantified after data collection.

Chlorophyll a fluorescence emission measurements have been performed following a method described by Melo et al. Initial (F₀), maximum (F_m), variable (F_v = F_m - F₀) and potential quantum yield of photosystem II (PSII = F_v/F_m), using the fluorometer model OS-30p+ (Optisci) was measured. The determination of the chlorophyll a, b and total index has been performed using a non-destructive method using a portable chlorophyll meter (ClorofiLOG®, model CFL 1030). The indexes have been calculated according to the Falker chlorophyll index (FCI).

Data have been submitted to analysis of variance and means have been compared by the Tukey test, at the 5% level of significance, in the cases of significance has performed polynomial regression analysis using the statistical program SAS University [11].

3. RESULTS AND DISCUSSION

In general, the concentration of *Meloidogyne javanica* applied to eggplants (*Solanum melongena* L.) in the present experimental conditions did not cause significant changes in growth variables (Table 1). Results suggest that eggplant is a species that presents high rusticity, presenting high tolerance to diseases.

Table 2. Mean values obtained for the variables of plant height (PH), stem diameter (SD), leaves number (LN), flower buds (FB), root dry mass (RDM), shoot dry mass (SDM), total dry matter (TDM) and Dickson quality index (DQI) in eggplant with nematode (WN) and eggplant without nematode (NN).

Sources of variation	Variables analyzed							
	PH	SD	LN	FB	RDM	SDM	TDM	DQI
WN	52.1 a	10.49 a	25.6 a	10.2 a	65.32 a	26.11 a	91.43 a	92.90 a
NN	48.1 a	10.45 a	22.2 a	8.2 a	42.84 a	25.35 a	68.19 a	69.36 a
CV (%)	10.53	9.90	18.47	32.96	30.21	12.67	21.67	21.71

Means followed by the same letters in the column do not differ from each other by the Tukey test up to 5% probability.

In general, formation of knots in eggplant roots occurs in a smaller size compared to other species, such as tomato (*Solanum lycopersicum*), chilli peppers (*Capsicum* spp) and pepper (*Capsicum annuum*) [7], suggesting that species tolerates up to certain population levels of nematodes, since when they infect roots, symptoms intensify on growth plants, which was not observed in this study.

Nóia et al. [12], evaluating the hostability of onion genotypes (*Allium cepa*) and eggplant, found that they were resistant to *M. javanica*, data that corroborate with the present study. Ribeiro et al. [13], found different values, showing that the 'Embu' eggplant is susceptible to this nematode, with a large number of knots and eggs in the roots. This divergence in relation to the present work is related to the resistance of this cultivar to the breed of *M. javanica* used.

It has been observed that the variables of initial, variable and maximum fluorescence, quantum efficiency of PSII, ratio between initial variable and initial fluorescence, chlorophyll a, chlorophyll b and total chlorophyll have not influenced by nematode infestation (Table 2).

Table 3. Mean values obtained for the variables of initial (F₀), variable (F_v), maximum (F_m) fluorescences, quantum efficiency of PSII (F_v/F_m), ratio (F_v/F₀), chlorophyll a (Ca), chlorophyll b (Cb) and total chlorophyll (tC) in eggplant with nematode (WN) and eggplant without nematode (NN).

Treatments	Variables analyzed							
	F ₀	F _v	F _m	F _v /F _m	F _v /F ₀	Ca	Cb	tC
WN	67.00 a	235.6 a	302.6 a	0.77 a	3.51 a	38.71 a	19.38 a	58.09 a
NN	72.20 a	234.0 a	306.2 a	0.76 a	3.34 a	38.16 a	20.78 a	59.02 a
CV (%)	13.71	6.02	2.74	4.01	15.52	3.60	14.96	6.94

Means followed by the same letter in the column do not differ by Tukey test up to 5% probability.

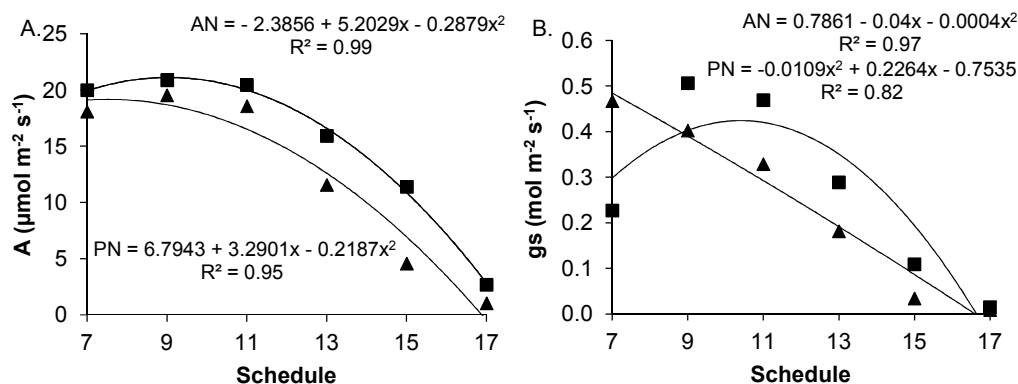
When the values of F_v/F_m are below 0.75 quantum⁻¹ electrons it can be highlighted that the plants are under stress, when it is between 0.75 and 0.85 quantum⁻¹ electrons, plant is performing its activities adequate photosynthetic properties [14]. Decrease in F_v/F_m values may be associated with the deviation of part of photons to the fluorescence pathway, rather than the photochemical pathway. If this happens, plant can reduce its photosynthetic processes. In this work, it has observed that, from these variables, plants are not under stress, which can be reaffirmed by the index of chlorophylls that has not been altered either.

The occurrence of this behavior can be due to defense mechanisms that occur in the plant, attenuating or preventing the attack of nematodes to promote physiological and biochemical disturbances in the plant. Plant resistance to nematodes of the *Meloidogyne* sp. family occurs through mechanisms that involve periods established during and after nematode penetration into the roots [15].

Plant resistance to nematode established as a series of characteristics developed by the plant in detriment to parasitism, and these can manifest themselves through mechanical barriers, physiological or chemical alterations, preventing the nematode from entering the tissues of the plant [16]. The same authors also claim that plant can promote changes on its life cycle and reproduction in response to nematode attack.

The induction of resistance in plants to nematode attack consists of a series of signals that act to alter the activity of the parasite. Some alterations are characterized by the interruption of parasitic cycle elongation and can act directly or indirectly on the hatching stimulus, the ability of the youngest to move and penetrate the roots of the host plant, and the maintenance of the nematode feeding site [17], this behavioral characteristic has facilitated the plant resistance to the attack of this pathogen.

Based on the obtained results, it has been observed that the values of CO₂ (A) assimilation, stomatal conductance (gs), internal carbon concentration (Ci) and transpiration (E) presented a quadratic response to the effect of different reading (Figure 1). Regarding the net assimilation of CO₂, it is observed that the highest values were observed in the first reading times, both in the presence and absence of nematodes, reaching maximum values at 9:00 am with 20.89 and 19.54 μmol CO₂ m⁻² s⁻¹, respectively, gradually decreasing throughout the day (Figure 1A).



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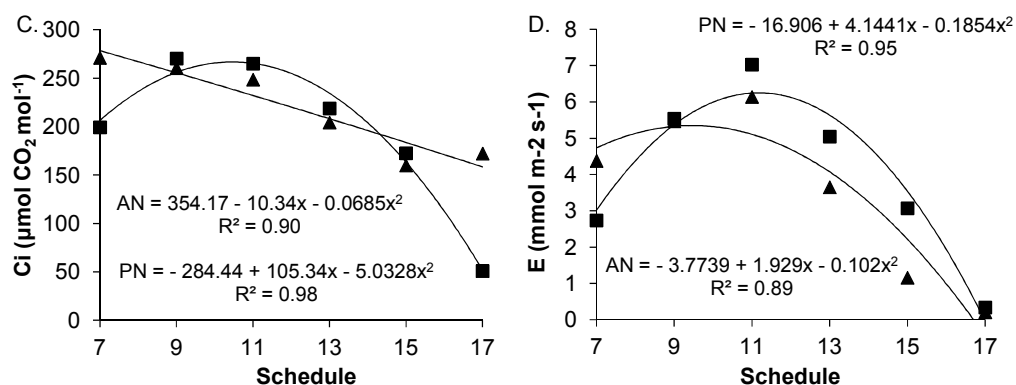


Figure 1. Daily variation of CO₂ net assimilation rate (A) (A), stomatal conductance (gs) (B), CO₂ internal concentration (Ci) (C) and transpiration (E) (D) in eggplant (*Solanum melongena* L.) plants in the presence (PN) and absence (AN) of nematodes (■) presence of nematoid (▲) absence of nematode

This behavior can be explained by the fact that the increase on temperature limits the CO₂ assimilation rate due to the reduction of CO₂ solubility and the ratio of carboxylation reactions to Rubisco oxidation in C3 plants, as in the case of eggplant [18]. It is also possible to observe that the plants cultivated in the presence of nematodes showed a higher photosynthetic rate, justifying the fact that the eggplant has been considered a rather rustic plant, being more tolerant to this type of stress than species of the same family, such as tomato and pepper [19]. The fact is that plant has increased its photosynthetic processes under the presence of the nematode can be related to the phenomenon of acclimatization to stress, which gives the plant a way to adjust its metabolic processes and cope with the stressful situations it is bestowed upon. Similar results have been observed by Mioranza [20], where the main symptoms caused by nematodes of the genus *Meloidogyne* in tomato plants were the increase of the photosynthetic rate.

For the stomatal conductance, it has been observed that plants conducted in the presence of nematodes reached maximum values at 9:00 am with 0.50 mol H₂O m⁻² s⁻¹, when conducted in the absence of these pathogens, presented the highest increase at 7:00 am, with 0.46 mol H₂O m⁻² s⁻¹, reducing at the following times (Figure 1B). This reduction can be due to the increase on temperature, which causes the stomatal closure and, consequently, the decrease of the transpiration rate, thus avoiding the loss of water.

According to Magalhães Filho et al. [21], plants have the stomatal closure as the first line of defense when subjected to some type of stress, which occurs both due to dehydration of the guard cells as well as by hormonal response. Endres [22] with *Annona squamosa* found similar results, where the stomatal conductance was higher in the first reading times, with decreases starting at 8:00 hours, a fact that according to the same author should be due to the increase in the vapor pressure deficit (VPD).

The internal CO₂ concentration follows the same stomatal conductance behavior, presenting a significant difference between the treatments, with maximum values of 270.32 and 270.95 μmol CO₂ mol⁻¹ for plants conducted with and without nematodes, respectively (Figure 1C). This behavior according to Silveira et al. [23], occurs because the stomatal opening is related to the diffusion of CO₂. Machado et al. [24] also show this change in orange tree, where the CO₂ input in the leaf mesophyll was lower as a function of the decrease in stomatal conductance.

Values for transpiration also showed a similar behavior to stomatal conductance, with maximum values of 7.03 and 6.14 mmol H₂O m⁻² s⁻¹ at 11:00 hours in plants with and without nematodes, respectively, decreasing hours after (Figure 1D). Machado et al. [24], affirm that the stomatal conductance is proportional to the perspiration, supporting the hypothesis that bigger the stomatal limitation, lower the transpiration rate.

These results are similar to those found by Messchmidt [25], stating that this stress can increase the production of abscisic acid (ABA) in the root system of the crop, and this signal is transmitted to the

aerial part, which will cause the stomatal closure, promoting a decrease in the rates of photosynthesis, transpiration and stomatal conductance.

Values for the instantaneous efficiency of carboxylation (iEC), water use efficiency (WUE), intrinsic water use efficiency (iWUE) and leaf temperature (LT) have also adjusted to the quadratic effect (Figure 2). For the carboxylation efficiency, results were similar to CO₂ assimilation and intracellular CO₂ concentration, with the highest increases being observed at 7:00 hours for plants conducted in the presence of nematodes, with 0.10 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and at 9:00 hours for nematode-free plants with 0.07 $\mu\text{mol m}^{-2} \text{s}^{-1}$, respectively (Figure 2A). Ferraz et al. [26] report that this behavior has occurred due to the increases in assimilation rates and intracellular CO₂. This efficiency of CO₂ fixation plays an important role for plant growth and aptitude [27].

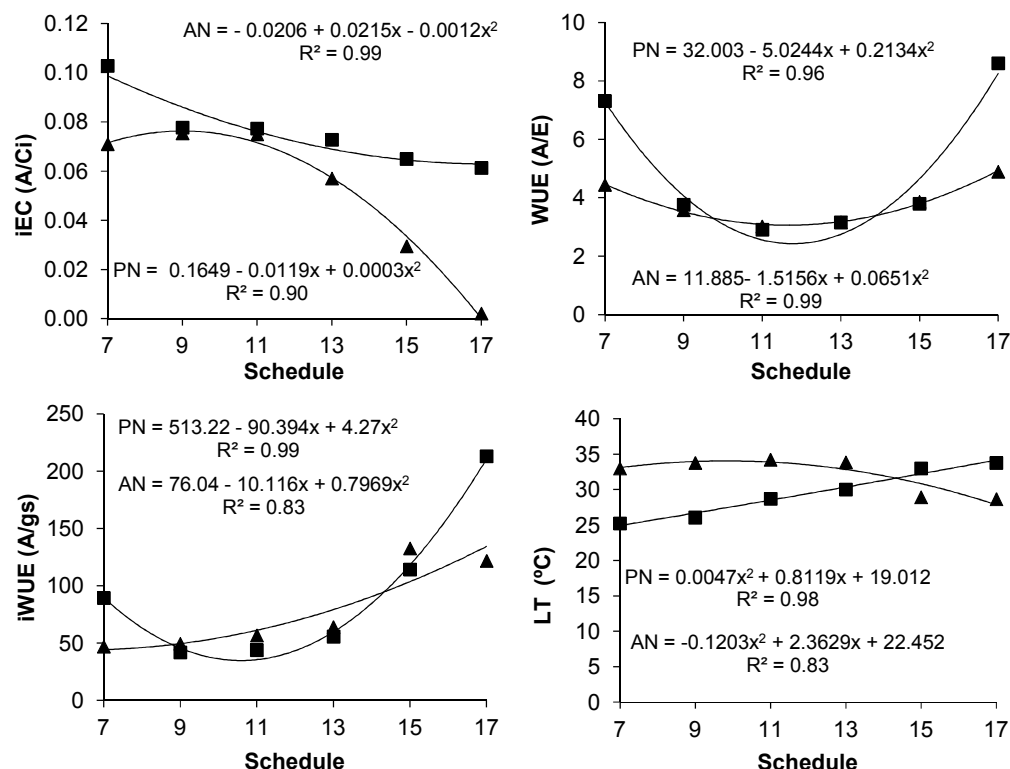


Figure 2. Daily variation of instantaneous carboxylation efficiency (iEC) (A), water use efficiency (WUE)(B), intrinsic water use efficiency (iWUE) (C) and leaf temperature (LT) (D) in eggplant (*Solanum melongena* L.) in the presence (PN) and absence (AN) of nematodes (■) presence of nematoid (▲) absence of nematode

Water use efficiencies behaved similarly throughout the day, with maximum increases in late afternoon (Figure 2B) and (Figure 2C), respectively. It can be a reflection of the stomatal closure observed during reading, Machado Filho et al. [28], working with papaya under field conditions also found values similar to the present study for the intrinsic efficiency on water use.

Foliar temperature had a distinct behavior between plants conducted with and without nematodes, and plants conducted in the absence of nematodes, on average, showed higher leaf temperatures in relation to the others (Figure 2D). This is an important factor to point it out, since it can be a limiting factor for the photosynthetic process, where very high leaf temperatures limit the activity of Rubisco [29]. Since at higher temperatures the oxygenase activity of the Rubisco has increased, in detriment to the carboxylase activity, thus increasing the process of photorespiration and loss of CO₂.

216 4. CONCLUSION

- 217 • The presence of *Meloidogyne javanica* did not influence the growth characteristics, blooms,
 218 quantum efficiency of PSII, chlorophyll a, b and total in eggplant (*Solanum melongena* L.). However, it
 219 promoted a bigger increase on net CO₂ assimilation and in the carboxylation efficiency in eggplant.
 220 • Therefore, it has affirmed that these plants have mechanisms of regulation of their photosynthetic
 221 activities, which gives them tolerance to the stress of *M. javanica*, causing no damage to their growth.
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