Nativa, Sinop, v. 10, n. 1, p. 90-94, 2022.
Pesquisas Agrárias e Ambientais
DOI: https://doi.org/10.31413/nativa.v10i1.13217
ISSN: 2318-7670

Morphology of tomato plants under nematode attack and salicylic acid application

Francisco Romário Andrade FIGUEIREDO[1]*, João Everthon da Silva RIBEIRO[2], Toshik Iarley da SILVA[3], Jackson Silva NÓBREGA[2], Marlenildo Ferreira MELO[1], Manoel Bandeira de ALBUQUERQUE[2], Guilherme Silva de PODESTÁ[2]

[1]Postgraduate Program in Fitotechnics, Federal Rural University of the Semi-Arid, Mossoró, RN, Brazil.
[2]Postgraduate Program in Agronomy, Federal University of Paraíba, Areia, PB, Brazil.
[3]Postgraduate Program in Fitotechnics, Federal University of Viçosa, Viçosa, MG, Brazil.
*E-mail: romarioagroecologia@yahoo.com.br
(ORCID: 0000-0002-4506-7247; 0000-0002-1937-0066; 0000-0003-0704-2046; 0000-0002-9538-163X; 0000-0001-7449-6916; 0000-0003-1871-0046; 0000-0003-1613-7178)

Recebido em 03/12/2021; Aceito em 04/03/2022; Publicado em 14/03/2022.

ABSTRACT: Root-knot nematodes are the main soil-dwelling phytopathogens that cause severe damages to plants, especially tomato plants. Exogenous application of salicylic acid (SA) can mitigate such pathogenicity. This work aimed to evaluate the growth of tomato plants submitted to *Meloidogyne javanica* population densities (PD) and application of SA. The experiment was a randomized block design, in an incomplete factorial scheme (central composite design), with five PD (0, 5815, 20000, 34184, and 40,000 eggs per pot) and five SA doses (0.0, 0.29, 1.0, 1.71, and 2.0 mM), with four replicates containing two plants each. Number of leaves, plant height, stem diameter, shoot dry mass, root dry mass and total dry mass, Dickson's quality index, leaf area, specific leaf area, specific leaf weight, root volume, absolute and relative growth rates for plant height, number of eggs, number of galls, and nematode reproduction factor were evaluated at 50 days after soil inoculation (DAI). Results showed the application of 0.97, 2.0, and 0.88 mM SA increased, respectively, the RGR, SLA and SLW. On the other hand, 0.91 and 0.93 mM SA decreased, respectively, the number of eggs and reproduction factor of nematodes. Also, *M. javanica* did not affect the growth of tomato plants until 50 DAI.
Keywords: *Meloidogyne javanica*; phytohormone; *Solanum lycopersicum*.

Morfologia do tomateiro sob ataque de nematoides e aplicação de ácido salicílico

RESUMO: Nematoides das galhas são uns dos principais patógenos de solo que causam danos severos nas plantas, especialmente em plantas de tomate. A aplicação exógena de ácido salicílico (AS) pode minimizar os efeitos desses patógenos. O objetivo deste trabalho foi avaliar o crescimento de plantas de tomate submetidas à densidades populacionais de *Meloidogyne javanica* (DP) e aplicação de AS. O delineamento em blocos casualizados em esquema fatorial incompleto (Composto Central de Box) com cinco DP (0, 5815, 20000, 34184 e 40000 ovos por planta) e cinco doses de AS (0.0, 0.29, 1.0, 1.71 e 2.0 mM), com quatro repetições e duas plantas por repetição foi utilizado. O número de folhas, altura de planta, diâmetro do caule, massa seca da parte aérea, raiz e total, índice de qualidade de Dickson, área foliar, área foliar específica, peso específico de folha, volume de raiz, taxas de crescimento absoluto e relativo para altura, número de ovos, número de galhas e fator de reprodução dos nematoides foram avaliados aos 50 dias após a inoculação do solo (DAI). A aplicação de 0.97, 2.0 e 0.88 mM de AS aumentam a taxa de crescimento relativo de altura, área foliar específica e peso específico de folhas, respectivamente. A aplicação de 0.91 e 0.93 mM de AS diminuem o número de ovos por grama de raiz e fator de reprodução, respectivamente. *M. javanica* não influenciou o crescimento de plantas de tomate até 50 DAI.
Palavras-chave: *Meloidogyne javanica*; fitohormônio; *Solanum lycopersicum*.

1. INTRODUCTION

Nematodes, especially those from the *Meloidogyne* genus, are the main soil-dwelling phytopathogens. They form galls on the roots of parasitized plants (VIGGIANO et al., 2014), causing stunted growth, wilting, leaf discoloration, and root deformation. *M. incognita* and *M. javanica* are the most harmful nematode species, depending on population density, crop susceptibility, soil type, and environmental conditions (SAUCET et al., 2016).

These phytoparasites cause severe damages in tomato plants (*Solanum lycopersicum* L.), one of the main vegetables consumed in Brazil, as a source of vitamins and minerals (PERVEEN et al., 2015). However, high nematode infestation at planting can cause up to 100% fruit production losses, in addition to reducing fruit quality (OLIVEIRA; ROSA, 2014). Chemical nematicides have been used to control these pathogens. However, frequent use of these chemicals may cause toxicity and contaminate the environment in addition to being ineffective and rising production costs (ESCUDERO et al., 2016). Thus, it becomes necessary to find effective products and techniques that minimize such effects.

Salicylic acid (SA), a phytohormone of phenolic origin that acts as a resistance inducer against biotic and abiotic stresses, is a promising alternative against nematode attack on plants (BORSATTI et al., 2015). SA was demonstrated as a resistance inducer in soybean under *Pratylenchus brachyurus* attack (LOPES et al., 2017) and as a resistance gene inducer in *Gynura aurantiaca* L. (CAMPUS et al., 2014).

Studying the effect of SA on the growth of tomato plants grown in soil infested by *M. javanica* is relevant due to the effectiveness of this phytohormone in inducing resistance (MOSTAFANEZHAD et al., 2014a). Thus, this work aimed to evaluate the growth of tomato plants submitted to *M. javanica* densities and application of salicylic acid.

2. 2. MATERIALS AND METHODS

2.1. Inoculum Preparation

Tomato plants (*Solanum lycopersicum* L. cv. Santa Clara) were grown in pots (2 dm^3 capacity) filled with soil and sand (2: 1 v / v) for 70 days to multiply and obtain the pathogen inoculum (*Meloidogyne javanica*). Tomato roots infected by nematodes were washed and crushed in a blender in 0.5% sodium hypochlorite solution (NaClO), under low rotation for 20 seconds. Then, the solution was filtered through 200 and 500 mesh sieves, respectively. The content of the 500 mesh sieve was washed in running water to eliminate NaClO then placed in a beaker to quantify the number of eggs under an optical microscope (HUSSEY; BARKER, 1973). The soil infestation was carried out at the time of transplanting according to the treatments.

2.2. Preparation of Salicylic Acid

Distilled water was used to prepare the salicylic acid (SA) doses. Three applications were performed at 15-day intervals: the first one immediately after transplanting and soil infestation, and the last one the day before the initial evaluation.

2.3. Conditions and Experimental Design

The experiment was carried out in a greenhouse at the Department of Crop and Environmental Sciences, Federal University of Paraíba (UFPB), Areia city, Paraíba State, Brazil.

Tomato seedlings (Santa Cruz Kada cultivar (Paulista), Isla®, Porto Alegre, Brazil) were produced in polyethylene trays with a commercial substrate (Basaplant®, Artur Nogueira, Brazil). When they reached 10 to 15 cm in height, the seedlings were transplanted into pots (5 dm^3 capacity) filled with a substrate composed of soil, sand, and cattle manure (3: 1: 1 v / v). The substrate was previously sterilized in an autoclave at 120ºC and 1 atm of steam pressure for two hours. The plants were daily irrigated to keeping substrate at field capacity. A substrate sample was taken for for physicochemical analysis (Embrapa, 2018): 7.8 pH; 85.55 and 693.60 mg kg^{-3} P and K, respectively; 0.23, 0.00, 2.91, 1.59, 6.50, and 6.50 cmolc dm^{-3} Na^+, $H^+ + Al^{3+}$, Ca^{2+}, Mg^{2+}, sum of bases (SB), and cation exchange capacity (CEC), respectively; and 22.21 g kg^{-1} organic matter (OM).

The experimental design was a randomized block, in an incomplete factorial scheme (central composite design), with five nematode population densities (0, 5815, 20000, 34184, and 40,000 eggs per pot) and five doses of salicylic acid (0.0, 0.29, 1.0, 1.71, and 2.0 mM), with four replicates containing two plants each, totalling nine combinations (MATEUS et al., 2001).

2.4. Analyzed Variables

The evaluations were carried out at 50 days after transplanting and soil inoculation (DAI), being measured the variables: number of leaves, plant height, stem diameter, shoot dry weight, root dry weight and total dry mass, Dickson's quality index (DICKSON et al. 1960) (Equation 1):

$$\mathrm{DQI} = \frac{\mathrm{TDM}/(\frac{\mathrm{PL}}{\mathrm{SD}})}{\mathrm{SDM}/\mathrm{RDM}} \quad (01)$$

where: DQI = Dickson's quality index, TDM = total dry mass, PL = plant length, SD = stem diameter, SDM = shoot dry mass and RDM = root dry mass.

Leaf area (BLANCO; FOLEGATTI, 2003) (Equation 2):

$$\mathrm{LA} = 0.347(\mathrm{LxW}) - 10.7 \quad (02)$$

where: LA = leaf area (cm^2), L = length (cm) and W = width (cm).

Specific leaf area and specific leaf weight (BENICASA, 2003) (Equation 3 and 4):

$$\mathrm{SLA} = \mathrm{LA}/\mathrm{LDM} \quad (03)$$
$$\mathrm{SLW} = \mathrm{LDM}/\mathrm{LA} \quad (04)$$

where: LDM = leaves dry mass.

Root volume (RV): determined with the support of a beaker, where the roots were submerged in a known volume of water.

Relative (RGRph) and absolute growth rates (AGRph) were determined according to Benicasa (2003) (Equation 5 and 6):

$$\mathrm{RGRph} = \frac{(\mathrm{InPh2} - \mathrm{InPh1})}{\mathrm{t2} - \mathrm{t1}} \quad (05)$$

$$\mathrm{AGRph} = \frac{(\mathrm{Ph2} - \mathrm{Ph}\ )}{\mathrm{t2} - \mathrm{t1}} \quad (06)$$

The evaluations were carried out every 15 days, in that: Ph1 = plant height (cm) at time t1, Ph2 = plant height (cm) at time t2 and ln = natural logarithm.

The number of eggs per gram of root (NE g^{-1}) was determined by counting, under an optical microscope, the total number of eggs in Petri dishes then divided by root fresh weight; the number of galls per gram of root (NG g^{-1}) was determined by counting the number of galls present in the root system; and the reproduction factor (RF) was obtained by the ratio between the final and initial population density.

2.5. Statistical analysis

Data were submitted to analysis of variance by the F test ($p < 0.05$) followed by polynomial regression analysis. All statistical analyses were performed in R software (R CORE TEAM, 2019).

3. RESULTS

Interaction between *M. javanica* population densities and SA doses was not significant. Also, the population densities did not affect the studied variables. On the other hand, SA doses affected specific leaf area, specific leaf weight and relative growth rate ($p<0.05$).

SA stimulated RGR_{PH}, SLW and SLA in the tomato plants (Figure 1). RGR_{PH} and SLW increased under up to 0.97 and 0.88 mM SA concentrations, reaching 0.100 cm cm^{-1} day^{-1} and 0.020 g cm^2 on average, respectively.

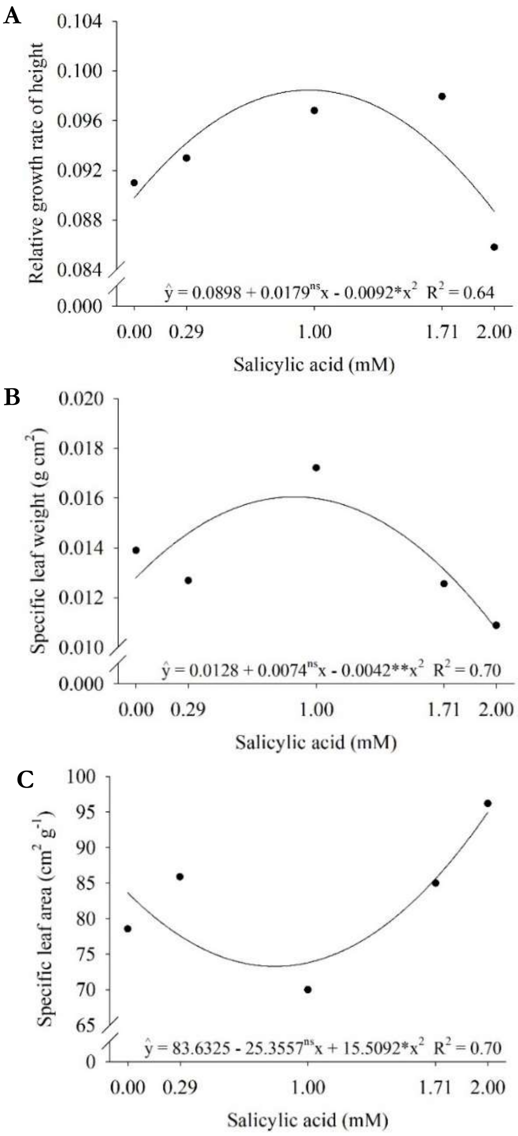

Figure 1. Relative growth rate for plant height (A), specific leaf weight (B), and specific leaf area (C) in tomato plants under salicylic acid application.

Figura 1. Taxa de crescimento relativo para altura de plantas (A), peso específico de folhas (B) e área foliar específica (C) em plantas de tomate sob aplicação de ácido salicílico.

Nematode population densities positively influenced the number of eggs (NE), number of galls (NG), and reproduction factor (RF). In turn, SA positively influenced NE and RF ($p<0.05$).

NG linearly increased with increasing inoculum concentration (Figure 2A). On the other hand, NE was higher at 23903 eggs per plant, decreasing afterwards (Figure 2B), while RF (20.01) was higher in 20079 eggs per plant (Figure 2C).

NE decreased by 67.5% while RF decreased by 46.4% under application of up to 0.91 and 0.93 mM SA, respectively, but increased after that (Figures 3A and 3B).

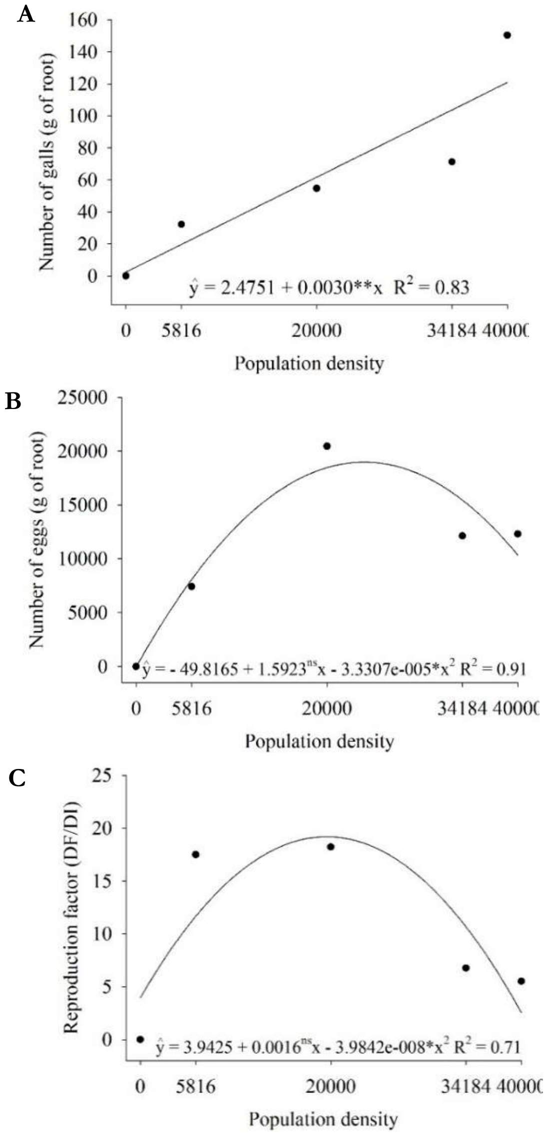

Figure 2. Number of galls (A) and eggs (B) per gram of root and reproduction factor (C) in tomato plants under population densities of *Meloidogyne javanica*.

Figure 2. Número de galhas (A) e ovos (B) por grama de raiz e fator de reprodução (C) em plantas de tomate sob densidades populacionais de *Meloidogyne javanica*.

4. DISCUSSION

PDs did not affect plant growth because the time (50 days) between the soil infestation and plant evaluation was not enough for the nematodes to reproduce and increase the infestation (ABRÃO; MAZZAFERA, 2001). However, different results were observed in cherry tomato (*S. lycopersicum* var. Cerasiforme) infested by nematodes. Regardless of population density, the parasitized site worked as a sink for photoassimilates resulting in reduced leaf expansion (BELAN et al., 2011).

In turn, SLA was higher at 2.0 mM dose, with a 13.5% increase compared to control. Results support that SA effects on plant growth vary according to the application form and plant species studied (EL-ESAWI et al., 2017). It has been shown that SA favors cell extension but limit cell division, denoting a reason for the effectiveness of this phytohormone (JAYAKANNAN et al., 2015).

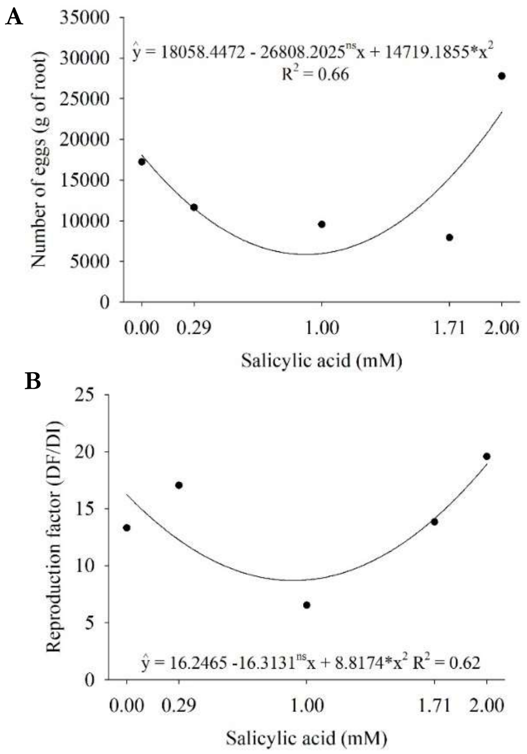

Figure 2. Number of eggs (A) and reproduction fator (B) in tomato plants under population densities of *Meloidogyne javanica*.
Figure 2. Número de ovos (A) e fator de reprodução (C) em plantas de tomate sob densidades populacionais de *Meloidogyne javanica*.

Similar results were observed in tomato plants treated with 0.75 mM SA, which increased the number of leaves, leaf area, and leaf dry weight (ARFAN et al., 2007). Such benefits on tomato growth occurred because SA significantly affects the plant physiological processes, like photosynthesis that can be induced or inhibited respectively by low and high SA concentrations used (KHAN et al., 2015). Also, the duration of exposure, plant species, age, and treated organ may influence the SA effects on plants (MIURA; TADA, 2014).

The linear increase in NG occurred because the inoculum amount directly affects the number and size of galls, thus increasing according to population density (KAYANI et al., 2017). In a short time, in plants under low infestation levels, the nematode population growth is exponential. However, as the population increases, competition for space and nutrients also increases among nematodes, which thus reduces their growth rate (CARNEIRO et al., 1999). It may explain why NE and RF reduced under higher population densities.

The beneficial effect of SA is due to this hormone induce defense mechanisms in plants, such as increasing production of resistance-related enzymes like phenylalanine ammonium lyase (PAL), peroxidase (POX) and polyphenol oxidase (PPO), and stimulating the accumulation of phenolic compounds (MOSTAFANEZHAD et al., 2014b). However, SA causes hormonal imbalance at high levels, becoming plants susceptible to pathogen attack. In tomato plants, the beneficial effect of this hormone is associated with the activation of SAR-related genes (MOSLEMI et al., 2016).

5. CONCLUSIONS

Salicylic acid applied at 0.97, 2.0 and 0.88 mM concentrations increases respectively the relative growth rate for plant height, specific leaf area, and specific leaf weight in tomato plants.

At 0.91 and 0.93 mM concentrations, SA reduces the number of eggs per gram of root and reproduction factor of nematodes, respectively.

Meloidogyne javanica does not influence tomato growth until 50 days after soil infestation under these experimental conditions.

6. ACKNOWLEDGMENTS

The authors thank the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and the Conselho National de Desenvolvimento Científico e Tecnológico (CNPq), Brazil, for granting the scholarships.

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