Morphology of tomato plants under nematode attack and salicylic acid application

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RESUMO: 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 40000 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

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 40000 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.

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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 (ESCUDEIRO 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...
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. This suspension of nematodes was 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. 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 keep the substrate at field capacity. A substrate sample was taken for physicochemical evaluation.

2.4. Analyzed Variables

The evaluations were carried out 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):

$$DQI = \frac{TDM}{RDM} - 10.7$$

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):

$$LA = 0.347(LxW) - 10.7$$

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):

$$SLA = \frac{LDM}{LA}$$

$$SLW = \frac{LDM}{LA}$$

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):

$$RGRph = \frac{(Ph2 - Ph1)}{t2 - t1}$$

$$AGRph = \frac{(Ph2 - Ph1)}{t2 - t1}$$

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.

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).

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).
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.

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