Silicon promotes the control of *Meloidogyne incognita* in lettuce by increasing ascorbic acid and phenolic compounds

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Research Article

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Abstract

The use of silicon (Si) has a physical barrier effect on plant tissues, decreasing nematode infection in different crops. Notwithstanding, research on lettuce crop is lacking, especially regarding the chemical mechanisms of action of this beneficial element. Therefore, this study evaluates the effect of Si supply on lettuce plants infested with 0, 6000, and 12000 eggs and second stage juveniles of *M. incognita*, both in the absence and in the presence of Si (2 mM) in the nutrient solution. Silicon increases phenolic compounds and ascorbic acid, reducing the *M. incognita* population and decreasing oxidative stress. It also increases chlorophyll index and the quantum efficiency of the photosystem II (FV/FM), favoring the growth and production of lettuce plants. The use of Si decreased the number of nematodes and affected their reproduction, decreasing the number of eggs and galls in the roots of lettuce plants, being yet another sustainable alternative for the control of *M. incognita*. The Si benefit would be due to the combined effect of the physical barrier and the chemical action from the increase in phenolic compounds and ascorbic acid in plant tissues, improving the physiological aspects of plants.

Key Messages

- The *Lactuca sativa* is a crop susceptible to the attack of root-knot nematode.
- The Si strengthens the defense system of this plant against *incognita*.
- Si has an effect on increasing the firmness of tissues acting as a physical barrier in control of *incognita*.
- Si supply decreases the reproduction rate of *incognita*.
- The Si a sustainable alternative, in control of root-knot nematode since its use can reduce applications with nematicides.

Introduction

Lettuce (*Lactuca sativa* L.) is among the most produced and consumed leafy vegetables in the world. It has high economic importance (Mou 2012) and health benefits for being a source of vitamins and antioxidant compounds (Aksakal et al. 2017), However, most cultivated lettuce cultivars show a certain degree of susceptibility to nematodes, especially to the root-knot nematode *Meloidogyne incognita*, which is known to decrease lettuce growth and yield (Wilcken et al. 2005; Dias-arieira et al. 2012). *Meloidogyne* species form root vesicles during infection, thickening roots and causing cell hyperplasia and hypertrophy. This induces gall formation(Ornat and Sorribas 2008) and impairs root growth, especially lateral root growth (Moens et al. 2009). Root damage, in turn, decreases water and nutrient uptake (Amaral et al. 2013).

Increased nematode infection significantly affects crop yield. In this sense, and considering global population growth, the absence of effective strategies to control nematode populations and infections has serious and aggravating consequences for sustainable agriculture (Sato et al. 2019).

For this reason, there is a need for further research to evaluate new ways of controlling nematodes, with the most promising being the use of silicon. This element can provide an integrated environmentally friendly strategy as an alternative to the extensive use of pesticides (Faiq et al. 2018), especially in short cycle crops such as lettuce, thus reducing risks to human health.

Research has demonstrated beneficial effects of silicon on the control of nematodes in several crops such as beet (Khan and Siddiqui 2020), rice (Zhan et al. 2018), coffee (Silva et al. 2010), banana (Oliveira et al. 2012), sugarcane (Guimarães et al. 2010) and oats (Asgari et al. 2018). However, there are no reports for lettuce crop.

Plants absorb silicon in the form of monosilicic acid (Liang et al. 2005). Transporters Lsi1 and Lsi6, belonging to the aquaporin family, are the main involved in the distribution of this element in root and shoot tissues (Mitani et al. 2011). Nonetheless, lettuce plants are not considered element accumulators, having higher root than shoot concentrations. When deposited in cells, silicon induces polymerization by forming opal crystals (Ma 2004). Studies evaluating the effects of Si on nematode control indicate that the element accumulates in the form of opal in the cell wall and in the extracellular spaces, forming a physical barrier that prevents penetration, feeding, and parasitism in the roots (Silva et al. 2010; Asgari et al. 2018; Khan and Siddiqui 2020).

In view of the need for a better understanding of Si mechanisms to control *M. incognita* in lettuce, we hypothesize that Si benefits would not be only due to the physical barrier, but also to the chemical action from the increase in phenolic compounds and ascorbic acid in tissues, improving the physiological aspects of plants. This hypothesis is promising due to two reasons. Firstly, research indicates that the isolated application of phenolic compounds (Oliveira et al. 2019) and synthesized ascorbic acid (Osman, 1993; Maareg et al., 2014) has a toxic effect and decreases the population of nematodes in the plant.

Secondly, there is no need to synthesize these compounds for application in the plant, since Si can naturally increase phenolic concentration, as observed in rice plants (Rodrigues et al. 2005) and ascorbic acid concentration, as observed in asparagus and kale plants (De Souza et al. 2019). Therefore, it is necessary to find out whether the natural increase of these compounds promoted by Si in the plant is enough to reduce nematode infection. Moreover, the additional effect of Si on increasing photosynthetic pigments (chlorophyll content) and flowering (Khan and Siddiqui 2020) may contribute to the growth of lettuce plants infected or not with *M. incognita*.

If the hypothesis of this study is correct, it will enable first knowledge on the effectiveness of the chemical mechanism induced by Si in strengthening the defense system of this plant against *M. incognita*. This benefit is important for sustainable cultivation of lettuce, given the global dissemination of this nematode.

This research evaluates whether the effect of Si supply on increasing the levels of phenolic compounds and ascorbic acid reduces the *M. incognita* population and decreases oxidative stress, increasing

chlorophyll content and the quantum efficiency of the photosystem II and thus favoring the growth and production of lettuce plants.

Material And Methods

2.1 Experimental conditions

Two experiments were carried out with lettuce cultivar Vanda under greenhouse conditions, between March and May 2020. During the experimental period, the relative air humidity varied largely ($69.4 \pm 13.5\%$), maximum temperature was $34.5 \pm 7.2^\circ\text{C}$, and minimum temperature was $16.4 \pm 5.7^\circ\text{C}$ (Fig. 1).

Figure 1. Maximum temperature (T Max.), minimum temperature (T Min.), maximum relative humidity (H Max.), and minimum relative humidity (H Min.) in the greenhouse. Indication of transplanting times in the first (E1) and second (E2) experiment, ionic strength of the culture solution (IS), and collection of lettuce plants.

Lettuce seeds were sown in styrofoam trays containing inert substrate. After sowing, irrigation was performed with distilled water. After emergence, seedlings were transplanted to 5 dm³ pots filled with sand previously washed with running water and deionized water; each pot contained two seedlings.

2.2 Treatment description

Two experiments were installed with a one week difference so as to observe repeatability. The experimental design comprised a 3x2 factorial scheme: control (without inoculation of *M. incognita*), 6000, and 12000 eggs and second stage juveniles (J2) of *M. incognita*, combined with the nutrient solution in the presence or absence of Si (2 mM). The treatments were arranged in randomized blocks with eight replicates.

After transplanting, the plants were irrigated with complete nutrient solution Hoagland and Arnon (1950) prepared with deionized water, with pH adjustment between 5.5 and 6.0 and with a change in the iron source from Fe-EDTA to Fe-EDDHA. During seedling preparation, after emergence, a nutrient solution was provided at 10% of the concentration indicated by the aforementioned authors. After transplanting, the nutrient solution was applied to the pots at a concentration equal to 20% for a period of 10 and 7 days in the first and second experiments, respectively. After this period, the concentration of the nutrient solution was increased to 50%, being applied for 21 days in both experiments. The concentration was then increased to 70%, being applied until the end of the experimental period. After seedling transplanting, silicon was supplied in the form of potassium silicate (128 g L^{-1} of Si; 126 g L^{-1} of K₂O, pH 12) along with the nutrient culture solution. Potassium chloride was used for potassium balance in the nutrient solution between treatments.

2.3 Nematode inoculum

Two days after transplant DAT (in both experiments) inoculation with *M. incognita*. For treatments with nematodes, the subpopulation *M. incognita* race 3 was used, recovered from cotton (*Gossypium hirsutum* L.) roots. The subpopulation was previously identified in the laboratory based on morphological characters of the perineal pattern (Taylor and Netscher, 1968), on the labial morphology of males (Eisenback et al., 1981), and on the isoenzymatic phenotype for esterase by authors Esbenshade and Triantaphyllou (1990) using a traditional BIO-RAD Mini Protean II vertical electrophoresis system.

The subpopulation was then inoculated into tobacco and cotton plants according to the North Carolina Differential Host Test. Hartman and Sasser (1985). The subpopulation was multiplied in tomato (*Lycopersicon esculentum* Mill.) cultivar Santa Cruz Kada under greenhouse conditions. After 45 days of inoculation, the plants were removed from the pots, and the roots were washed and crushed in a blender with 0.5% sodium hypochlorite solution. The suspension was then passed through a 200-mesh sieve (0.074 mm opening) over a 500-mesh sieve (0.025 mm openings). The eggs and juveniles retained in the 500-mesh sieve were washed and collected in aqueous suspension in a 500 mL beaker.

In both experiments, the concentration of the suspension was determined and adjusted to 1200 and 2400 eggs and second stage juveniles (J2) of *M. incognita* mL⁻¹ using the Peters counting chamber (Southey 1970). One day after transplanting the lettuce seedlings, 5 mL of the suspension was inoculated, equivalent to 6000 and 12000 eggs and J2 of *M. incognita* per seedling, which corresponds to a high level of weed infestation, capable of causing economic damage to susceptible cultivars. *Abelmoschus esculentus* was used as a standard for inoculum viability.

Analysis

2.4.1 Electrolyte leakage index

Five leaf discs were collected from the first fully developed leaf at 41 DAT and 55 DAT in the first and second experiments, respectively. The discs were placed in a beaker with 20 mL of deionized water, at room temperature, for 2 h. After this period, initial electrical conductivity (EC1) was determined using a bench conductivity meter (TDS-3 digital meter). Subsequently, the samples were autoclaved for 20 minutes at a temperature of 121°C. After cooling, a new reading of the electrical conductivity was performed to determine final electrical conductivity (EC2). Electrolyte leakage was then calculated according to the formula proposed by authors Dionisio-Sese and Tobita (1998).

$$EE = \frac{EC1}{EC2} \times 100$$

2.4.2 Leaf firmness index

On harvest day, the leaf firmness was measured using a digital penetrometer with an 8 mm tip to apply a force ranging from 5 to 200 N ± 1 N (Impac, model IP-90DI, São Paulo, SP, Brazil). Three leaves per plant

were used, and three measurements were taken in the center of each leaf, with values expressed in Newton (N) according to Chitarra and Chitarra (2005).

2.4.3 Estimation of chlorophyll

Chlorophyll was determined using the ClorofiLOG chlorophyll meter (CFL 1030, FALKER), with the sensor performing measurements in contact with newly developed leaves, at 40 DAT and 54 DAT in the first and second experiments.

2.4.4 Photosystem II efficiency (Fv/Fm)

Chlorophyll fluorescence was measured according to the methodology proposed by authors Lichtenthaler et al. (2005) at 40 DAT and 54 DAT in the first and second experiments, using a saturation pulse fluorometer (Opti-Sciences® - Os30P+). Before the measurement, the first fully developed leaf used for determination was adapted to the dark for 30 minutes. The reading considered maximum and variable fluorescence. From these data, photosystem II (PSII) (FV/FM) quantum efficiency was calculated by the ratio between maximum fluorescence and variable fluorescence.

2.4.5 Ascorbic acid (AsA)

For the determination of ascorbic acid (AsA), two leaves were used. The first leaf was from the region with newly developed leaves; the second leaf was from the middle region of the plant, with fully developed leaves. The AsA content was quantified by titration with a 2,6-dichlorophenol-indophenol sodium solution (Tillman's reaction), with results expressed in mg of ascorbic acid per 100 g FM (fresh matter) according to AOAC methodology (1980) at 42 DAT and 56 DAT in the first and second experiments.

2.4.6 Total phenols

For total phenols, extraction and reading followed the methodology proposed by authors Singleton and Rossi (1965) at 41 DAT and 55 DAT in the first and second experiments. Hence, 0.1 g of fresh leaves (first fully developed leaves) were collected and subsequently placed on a 15 mL Falcon tube. The sample was then covered with aluminum paper and diluted in concentrated methanol in a water bath at 25°C for 3 hours. For the colorimetric reaction, 1 mL of the filtrated extract was transferred to another 15 mL Falcon tube, also covered with aluminum paper. The volume was completed with 10 mL of water and 0.5 mL of 2 N Folin-Ciocalteu, and the solution was allowed to rest for 3 minutes. After that, 1.5 mL of 20% sodium carbonate solution was added and left to react for 2 hours. Finally, absorbance was read in a spectrophotometer at 765 nm. Control samples were elaborated following the described procedures, with the exception of the fresh material. To achieve zero in the equipment, we used methanol. Total phenolic content was calculated as Equivalent Acid Gallic (EAG), the results are expressed in g EAG 100 g⁻¹ FW.

2.4.7 Leaf area, shoot fresh and dry matter, and root dry matter

After shoot collection, at 43 DAT and 57 DAT in the first and second experiments, leaf area was measured using AreaMeter® (L-3100, Li-Cor, USA). Subsequently, shoot samples were weighed to determine fresh matter.

Shoots and roots were then washed in running water, detergent solution (0.1% Extran®, v/v), acid solution (0.3% HCl, v/v), and deionized water. Then, the material was packed in paper bags and dried in a forced air circulation oven at 65 ± 5 °C until reaching constant shoot dry matter and root dry matter.

2.4.8 Estimation of nematode population and number of galls

The number of galls on lettuce roots was manually and visually counted. To determine the number of eggs and different stages of development of *M. incognita*, were according to the described methods Hussey and Barker (1973) extraction technique and the method of authors were used. (Coolen and D'Herde 1972). Afterwards, the nematode population in the samples was estimated using a photonic microscope, with the aid of the Peters counting chamber (Southey 1970). For root population, the reproduction factor (RF) was determined by the quotient between the final and initial nematode population ($RF = Pf/Pi$).

2.4.8 Silicon content analysis

After weighing shoots and roots, the material was ground in a Wiley mill, and chemical analysis was performed to determine Si content. For this, wet-alkaline digestion of the plant material was carried out in the presence of NaOH and H₂O₂ in an oven at 90°C, as described by authors Kraska and Breitenbeck (2010) Then, Si colorimetric reading was performed by reacting the sample with ammonium molybdate in the presence of hydrochloric acid and oxalic acid, as described by author Korndörfer et al. (2004). The results of Si content and dry matter enabled the calculation of Si accumulation in the leaves and roots of plants.

2.5 Statistical analysis

The data were submitted to analysis of variance by the F test and, when significant, to the means comparison test (Tukey) at 5% probability. Statistical analyses were performed using the statistical software SAS 298 Version 9.1. It was not necessary to transform the data to meet the statistical model.

Results

3.1 Si accumulation and firmness index

In the presence of Si, control lettuce plants and those with a nematode population of 6000 eggs and J2 of *M. incognita* in the first and second experiments had the highest shoot Si accumulation (Fig. 2 (A) and (B)). However, in the first experiment, the control treatment had the highest root Si accumulation, not differing from the treatment with 12000 eggs and J2 of *M. incognita*. Noteworthy, in the second experiment, root Si accumulation was higher in the control treatment (Fig. 2 (C) and (D)). In the absence

of Si, the nematode population did not affect the accumulation of this element in the plants of the two experiments.

In both experiments, Si application increased the accumulation of this element in the roots and shoots of lettuce plants with different populations of nematodes (0, 6000, and 12000 eggs and J2 of *M. incognita*) (Fig. 2 (A, B, C,D)).

M. incognita populations correlated with Si for firmness index only in the first experiment (Fig. 2 (E)). The firmness index of lettuce leaves in the absence of Si did not differ either in control plants or in those with 6000 and 12000 eggs and J2 of *M. incognita* in the first experiment. However, in the second experiment, plants without Si and with nematode populations equal to 6000 and 12000 eggs and *M. incognita* J2 showed lower leaf firmness indexes (Fig. 2 (F)). In both experiments, Si application increased the firmness index of lettuce leaves grown with different nematode populations (0, 6000, and 12000 eggs and J2 of *M. incognita*).

Figure 2. Si accumulation in the shoots (A and B) and roots (C and D) of lettuce plants (cv. Vanda) from the first and the second experiments, grown in pots with sand inoculated with populations (P) of 0, 6000, and 12000 eggs and J2 of *M. incognita* per pot, which received nutrient solution in the absence (-Si) and in the presence of Si (+Si). ** and *: significant at 1 and 5% probability, respectively, by the F test. ^{ns}: not significant by the F test. Lower case letters show differences in relation to populations, and upper case letters in relation to silicon. Bars represent the standard error of the mean. n=8.

3.2 Number of galls, eggs, and adults and reproduction factor

The number of galls and eggs of *M. incognita* in lettuce roots, as well as the reproduction factor of the nematodes in the roots, depend on the interaction between populations and silicon (Fig. 3).

In the presence or absence of Si, the increase in nematode population increased the number of galls on the plants of the two experiments (Fig. 3 (A) and (B)). In both experiments, Si application decreased the number of galls on plants grown with both populations of nematodes.

In the presence or absence of Si, in both experiments, using nematode populations of 6000 and 12000 eggs and J2 of *M. incognita* instead of control plants increased the number of nematodes in lettuce roots and the number of eggs (Fig. 3 (C, D, E, F)). In both experiments, Si addition decreased the number of *M. incognita* in lettuce roots and the number of eggs on plants cultivated with the two nematode populations.

In the absence or presence of Si, in both experiments, inoculation with 6000 and 12000 eggs and J2 of *M. incognita* increased the root reproduction factor in relation to control plants (Fig. 3 (G) and (H)). In both experiments, Si application in the nutrient solution decreased the reproduction rates of *M. incognita* in

Figure 3. Number of galls in the first (A) and second experiment (B), number of *M. incognita* (J2 - second stage juveniles; J3 and J4 - third and fourth stage juveniles; MF - mature female) in lettuce roots in the first (C) and second experiment (D), eggs of *M. incognita* in lettuce roots in the first (E) and second experiment (F), and reproduction factor of *M. incognita* in lettuce roots in the first (G) and second experiment (H). All experiments used the lettuce cv. Vanda, grown in pots with sand inoculated with populations (P) of 0, 6000, and 12000 eggs and J2 of *M. incognita* per pot, which received nutrient solution in the absence (-Si) and in the presence of Si (+Si). ** and *: significant at 1 and 5% probability, respectively, by the F test. ^{ns}: not significant by the F test. Lower case letters show differences in relation to populations, and upper case letters in relation to silicon. Bars represent the standard error of the mean. n=8.

3.3 Electrolyte leakage, phenols, and ascorbic acid

Nematode populations correlated with Si for electrolyte leakage and phenol and AsA concentration in both experiments (Fig. 4). In the absence of Si, inoculation with 6000 and 12000 eggs and J2 of *M. incognita* increased electrolyte leakage rate in both experiments. Silicon addition to the nutrient solution decreased electrolyte leakage in plants grown with both nematode populations.

In the absence or presence of Si, in both experiments, inoculation with nematode populations decreased phenol and AsA concentration. In both experiments, Si application increased phenol and AsA concentration in all nematode populations under study.

Figure 4. Electrolyte leakage index from the first (A) and the second experiment (B), total phenols from the first (C) and the second experiment (D), ascorbic acid from the first (E) and the second experiment (F). All experiments used the lettuce cv. Vanda, grown in pots with sand inoculated with populations (P) of 0, 6000, and 12000 eggs and J2 of *M. incognita* per pot, which received nutrient solution in the absence (-Si) and in the presence of Si (+Si). ** and *: significant at 1 and 5% probability, respectively, by the F test. ^{ns}: not significant by the F test. Lower case letters show differences in relation to populations, and upper case letters in relation to silicon. Bars represent the standard error of the mean. n=8.

3.4 Chlorophyll and PSII quantum efficiency (Fv/Fm)

Nematode populations correlated with Si for total chlorophyll index (Chl *a + b*) and PSII quantum efficiency of lettuce leaves (Fig. 5). In the absence or presence of Si, inoculation with 6000 and 12000 eggs and J2 of *M. incognita* decreased leaf chlorophyll index (Chl *a + b*) in both experiments, except in plants from Experiment 1 that received Si (Fig. 5 (A) and (B)). In both experiments, Si application increased chlorophyll index (Chl *a + b*) in all nematode populations under study.

In the absence or presence of Si, in both experiments, the increase in nematode populations, with 6000 and 12000 eggs and J2 of *M. incognita*, decreased PSII efficiency (Fv/Fm), except in plants from Experiment 2 that did not receive Si (Fig. 5 (C) and (D)). In both experiments, Si application increased PSII efficiency (Fv/Fm) in all nematode populations under study.

Figure 5. Total chlorophyll ($a + b$) of the first (A) and second experiment (B) and quantum efficiency of photosystem II of the first (FV / FM) (C) and the second experiment (D). All experiments used the lettuce cv. Vanda, grown in pots with sand inoculated with populations (P) of 0, 6000, and 12000 eggs and J2 of *M. incognita* per pot, which received nutrient solution in the absence (-Si) and in the presence of Si (+Si). ** and *: significant at 1 and 5% probability, respectively, by the F test. ^{ns}: not significant by the F test. Lower case letters show differences in relation to populations, and upper case letters in relation to silicon. Bars represent the standard error of the mean. n=8.

3.5 Leaf area, number of leaves, and shoot fresh matter

Nematode populations correlated with Si for leaf area, number of leaves, and shoot fresh matter in the first experiment (Fig. (6)). In the absence or presence of Si, inoculation with 12000 eggs and J2 of *M. incognita* decreased leaf area, number of leaves, and shoot fresh matter of lettuce plants, but only in experiment 1 (Fig. 6 (A, C and E)).

In both experiments, Si addition to the nutrient solution increased leaf area, number of leaves, and shoot fresh matter of the plants in all nematode populations under study.

Figure 6. Leaf area of the first (A) and second experiment (B), number of leaves first (C) and second experiment (D), fresh matter shoot of the first (E) and second experiment (F), photo of the shoot of the first (G) and the second experiment (H). All experiments used the lettuce cv. Vanda, grown in pots with sand inoculated with populations (P) of 0, 6000, and 12000 eggs and J2 of *M. incognita* per pot, which received nutrient solution in the absence (-Si) and in the presence of Si (+Si). ** and *: significant at 1 and 5% probability, respectively, by the F test. ^{ns}: not significant by the F test. Lower case letters show differences in relation to populations, and upper case letters in relation to silicon. Bars represent the standard error of the mean. n=8.

3.6 Shoot and root dry matter

Shoot dry matter depends on the interaction between nematode populations and Si, which was restricted to the first experiment (Fig. 7 (A)). In the absence or presence of Si, inoculation with 12000 eggs and J2 of *M. incognita* decreased shoot dry matter in the first experiment (Fig. 7 (A)), and did not change root dry matter in any of the experiments (Fig. 7 (C) and (D)).

In both experiments, Si addition to the nutrient solution increased shoot dry matter (Fig. 7 (A) and (B)) and root dry matter of lettuce plants in all nematode populations under study (Fig. 7 (C), (D)).

Figure 7. Dry matter of shoot of the first (A) and second experiment (B) and dry matter of root of the first (C) and second experiment (D). All experiments used the lettuce cv. Vanda, grown in pots with sand inoculated with populations (P) of 0, 6000, and 12000 eggs and J2 of *M. incognita* per pot, which received nutrient solution in the absence (-Si) and in the presence of Si (+Si). ** and *: significant at 1 and 5% probability, respectively, by the F test. ^{ns}: not significant by the F test. Lower case letters show

differences in relation to populations, and upper case letters in relation to silicon. Bars represent the standard error of the mean. n=8.

Discussion

Sedentary endoparasites like *M. incognita* enter the plant through the root elongation zone and migrate to the cortex, as this region lacks cell wall reinforcements (Abad et al. 2008). In the experiments, we showed that increased parasite inoculation in plants with or without Si increased the number of galls and different stage specimens of *M. incognita* in lettuce roots, also increasing the number of eggs and the reproduction factor (Fig. 3). This indicates that lettuce plants are susceptible to *M. incognita*, a fact widely reported in the literature (Franchin et al., 2018; Souza et al., 2019).

Nematode infection in lettuce plants correlates with the greater availability of food for nematodes (Mahalik and Sahoo 2016), causing biological damage to plants (Amaral et al. 2013). Inoculation of 6000 or 12000 eggs and J2 of *M. incognita* caused plant stress as it increased the rate of electrolyte leakage due to the decrease in the content of antioxidant compounds (phenolic compounds and ascorbic acid) in the plants of the two experiments (Fig. 4).

In addition, oxidative stress has worsened because during tissue penetration the nematode releases secretions such as degrading enzymes and cell wall modifying proteins (Jones et al. 2013; Holbein et al. 2016). which weaken the tissue structure by increasing the production of reactive oxygen species (ROS) and lipid peroxidation (Holbein et al. 2016). This stress decreased chlorophyll content in the two experiments, except in plants from Experiment 1 with Si. However, chlorophyll fluorescence decreased in the two experiments, both in the presence and absence of Si (Fig. 5).

This physiological damage caused by nematodes that absorb water and nutrients from the plant resulted in losses in plant growth given the decrease in leaf area, number of leaves, fresh matter (especially in Experiment 1) (Fig. 6), and shoot dry matter (in both experiments), except in plants from Experiment 2 with Si (Fig. 7).

This shows the need to expand measures to sustainably control this nematode from the use of silicon. It is important to highlight the capacity of this crop to absorb this beneficial element, which can lead to promising results and benefits for nematode-parasitized plants.

The cultivation of lettuce plants with nutrient solution containing Si (2 mM) was sufficient to increase the uptake and consequently the accumulation of this element in the shoots and roots of plants from the two experiments (Fig. 1). Leaf Si in experiments 1 and 2 reached 1.2 and 1.1 g kg⁻¹, respectively (data not shown), in the plants that received Si. This indicates that lettuce does not accumulate this element since its leaf content is less than 5 g kg⁻¹ (Ma and Yamaji 2006).

This group of plants restricts absorption and transport of Si to the shoots (Pontigo et al. 2015), with

accumulated more in the roots (11.4 and 11.6 mg per plant) than in the shoots (6.2 and 7.7 mg per plant) of lettuce plants (experiments 1 and 2, respectively) (Fig. 2 (A, B, C, D)).

The increase in Si uptake by lettuce plants was sufficient to decrease the number of *M. incognita* galls in the roots, the number of eggs, and the reproduction factor in the two nematode populations from both experiments. The benefits of Si in decreasing infection and parasitism of *M. incognita* in lettuce plants would be the result of the combination of different plant mechanisms.

The most well-known mechanism of action of Si in the control of nematodes in the plant is the formation of a double silica layer on the cell wall, improving lignification of epidermal cells (Inanaga and Okasaka 1995) and making the cell wall more rigid and less susceptible to parasite penetration and enzymatic degradation (Faiq et al. 2018; Khan and Siddiqui 2020). The physical barrier effect of Si was evidenced in the two experiments on plants with different nematode populations due to the increase in the firmness index of the plant tissue that received the beneficial element (Fig. 2 (E) and (F)), a fact reported by other authors (Emad et al. 2017; Artyszak 2018). This silicon-promoted physical barrier makes it difficult for the stylus to penetrate, reducing the number of galls and the population and their multiplication in the roots (Silva et al. 2010; Khan and Siddiqui 2020).

In addition to the physical benefits of Si in decreasing *M. incognita* infection in lettuce plants, the endogenous chemical effect was unprecedented due to the increased content of phenolic compounds (Fig. 4 (C) and (D)) and AsA (Fig. 4 (E) and (F)) in the plants. This effect of Si on the endogenous increase in AsA was also seen in chard and cabbage plants (Souza et al., 2019), while the increase in phenolic compounds was observed in wheat plants (Ma et al. 2016). This is because Si can activate genes and signals for the biosynthesis of these defense compounds in a process called acquired systemic resistance (Fawe et al. 2001).

It should be noted that the relationships of these compounds with nematicidal action were verified only in synthesized products. Therefore, there are reports of exogenous phenolic compounds increasing the mortality of second stage juveniles and decreasing the number of galls of *M. incognita* in tomato (Oliveira et al. 2019), and reports of the use of AsA in tomato (Osman 1993) and beet (Maareg et al., 2014). These compounds play an important role in the host-parasite interaction (Arrigoni 1979). In fact, these authors add that exogenous application of AsA in susceptible plants inhibits the invasion of nematodes and can transform susceptible plants into tolerant ones.

The benefits of Si in nematode-infected plants also correlated with physiological aspects from the decrease in the rate of electrolyte leakage, which increased chlorophyll ($a + b$), and the efficiency of chlorophyll fluorescence, indicating attenuation of the oxidative stress of plants. The effect of Si on oxidative stress attenuation has been reported in rapeseed and mustard (Ashfaque et al. 2017; Hasanuzzaman et al. 2017), in which Si increased antioxidant compounds such as phenols in the plants (Shahnaz et al. 2011; Hajiboland et al. 2018). Study performed Khan and Siddiqui (2020) reported the effect of Si on both the increase in chlorophyll content and the FV/FM of beet plants inoculated with *M.*

incognita. Other authors reported these effects for plants without nematode infection (Song et al. 2014; Hussain et al. 2016; Maghsoudi et al. 2016; Asgari et al. 2018).

The improvement of physiological aspects in nematode-infected plants that received Si increased plant growth. This can be seen visually (Fig. 6 (G) and (H)) from the increase in leaf area (Fig. 6(A) and (B)), number of leaves (Fig. 6 (C) and (D)), and fresh (Fig. 6 (E) and (F)) and dry matter of shoots and roots (Fig. 7 (A, B, C, D)) of lettuce plants from the two experiments.

The results of this research allow us to accept the hypothesis that the benefit of Si in decreasing the infection of *M. incognita* in lettuce plants would be due not only to the physical barrier, but also to the chemical action from the increase in phenolic compounds and ascorbic acid in plant tissues, improving the physiological aspects of plants.

The study also showed that Si was important in lettuce crop even in plants without nematode infestation (control), given the improvement of physiological aspects reflecting crop growth variables. The beneficial effects of Si on the growth of stress-free lettuce plants are well documented (Voogt and Sonneveld 2001; Galati et al. 2015; Alves et al. 2020).

The research proposes Si supply at a concentration of 2 mM for cultivation of lettuce plants as an additional alternative for sustainable control of *M. incognita* since it induces the defense mechanisms of plants.

Conclusion

The use of Si in the cultivation of lettuce plants is another sustainable alternative for the control of *M. incognita*. The study showed that the Si benefit would be due to the combined effect of the physical barrier and the chemical action from the increase in phenolic compounds and ascorbic acid in plant tissues, improving the physiological aspects of plants.

Declarations

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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.

AUTHOR CONTRIBUTIONS

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RMP designed research. **TASA, DLS, LFLT** and **RJF** conducted experiments. **PLMS** contributed analytical tools. **TASA, DLS** and **LFLT** wrote the manuscript. All authors read and approved the manuscript.

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Figures

Figure 1

Maximum temperature (T Max.), minimum temperature (T Min.), maximum relative humidity (H Max.), and minimum relative humidity (H Min.) in the greenhouse. Indication of transplanting times in the first (E1) and second (E2) experiment, ionic strength of the culture solution (IS), and collection of lettuce plants.

Figure 2

Si accumulation in the shoots (A and B) and roots (C and D) of lettuce plants (cv. Vanda) from the first and the second experiments, grown in pots with sand inoculated with populations (P) of 0, 6000, and 12000 eggs and J2 of *M. incognita* per pot, which received nutrient solution in the absence (-Si) and in the presence of Si (+Si). ** and *: significant at 1 and 5% probability, respectively, by the F test. ns: not

significant by the F test. Lower case letters show differences in relation to populations, and upper case letters in relation to silicon. Bars represent the standard error of the mean. n=8.

Figure 3

Number of galls in the first (A) and second experiment (B), number of *M. incognita* (J2 - second stage juveniles; J3 and J4 - third and fourth stage juveniles; MF - mature female) in lettuce roots in the first (C)

lettuce roots in the first (E) and second experiment

(F), and reproduction factor of *M. incognita* in lettuce roots in the first (G) and second experiment (H). All experiments used the lettuce cv. Vanda, grown in pots with sand inoculated with populations (P) of 0, 6000, and 12000 eggs and J2 of *M. incognita* per pot, which received nutrient solution in the absence (-Si) and in the presence of Si (+Si). ** and *: significant at 1 and 5% probability, respectively, by the F test. ns: not significant by the F test. Lower case letters show differences in relation to populations, and upper case letters in relation to silicon. Bars represent the standard error of the mean. n=8.

Electrolyte leakage index from the first (A) and the second experiment (B), total phenols from the first (C) and the second experiment (D), ascorbic acid from the first (E) and the second experiment (F). All experiments used the lettuce cv. Vanda, grown in pots with sand inoculated with populations (P) of 0, 6000, and 12000 eggs and J2 of *M. incognita* per pot, which received nutrient solution in the absence (-Si) and in the presence of Si (+Si). ** and *: significant at 1 and 5% probability, respectively, by the F test. ns: not significant by the F test. Lower case letters show differences in relation to populations, and upper case letters in relation to silicon. Bars represent the standard error of the mean. n=8.

Figure 5

Total chlorophyll (a + b) of the first (A) and second experiment (B) and quantum efficiency of photosystem II of the first (FV / FM) (C) and the second experiment (D). All experiments used the lettuce cv. Vanda, grown in pots with sand inoculated with populations (P) of 0, 6000, and 12000 eggs and J2 of *M. incognita* per pot, which received nutrient solution in the absence (-Si) and in the presence of Si (+Si).

Lower case letters show differences in relation to populations, and upper case letters in relation to silicon. Bars represent the standard error of the mean. n=8.

Figure 6

Leaf area of the first (A) and second experiment (B), number of leaves first (C) and second experiment (D), fresh matter shoot of the first (E) and second experiment (F), photo of the shoot of the first (G) and

Loading [MathJax]/jax/output/CommonHTML/fonts/TeX/fontdata.js cv. Vanda, grown in pots with sand

inoculated with populations (P) of 0, 6000, and 12000 eggs and J2 of *M. incognita* per pot, which received nutrient solution in the absence (-Si) and in the presence of Si (+Si). ** and *: significant at 1 and 5% probability, respectively, by the F test. ns: not significant by the F test. Lower case letters show differences in relation to populations, and upper case letters in relation to silicon. Bars represent the standard error of the mean. n=8.

Figure 7

Dry matter of shoot of the first (A) and second experiment (B) and dry matter of root of the first (C) and second experiment (D). All experiments used the lettuce cv. Vanda, grown in pots with sand inoculated with populations (P) of 0, 6000, and 12000 eggs and J2 of *M. incognita* per pot, which received nutrient solution in the absence (-Si) and in the presence of Si (+Si). ** and *: significant at 1 and 5% probability, respectively, by the F test. ns: not significant by the F test. Lower case letters show differences in relation to populations, and upper case letters in relation to silicon. Bars represent the standard error of the mean.

n=8

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