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

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Received: 2 February 2021 / Revised: 7 December 2021 / Accepted: 9 December 2021 / Published online: 8 January 2022
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Abstract
Silicon (Si) has a physical barrier effect on plant tissues, decreasing nematode infection in different crops. Notwithstanding, research on lettuce is lacking, especially regarding the chemical mechanisms of action of this beneficial element. This study evaluated the effect of Si supply on lettuce plants infested with 0, 6000, and 12,000 eggs and second stage juveniles of *Meloidogyne incognita*, both in the absence and in the presence of Si (2 mM) in the nutrient solution. Silicon increased phenolic compounds and ascorbic acid, reducing *M. incognita* population and decreasing oxidative stress. The element also increased chlorophyll content and the quantum efficiency of photosystem II (FV/FM), favoring lettuce growth and production. The use of Si decreased the number of nematodes and affected their reproduction, decreasing the number of eggs and galls on lettuce roots. This indicates that Si may serve as a sustainable alternative for the control of *M. incognita*. The benefit of using Si appears to be due to the combined effect chemical action from the increase in phenolic compounds and ascorbic acid in plant tissues, improving plant physiology.

Graphical abstract

**Keywords** *Lactuca sativa* · Biotic stress · Beneficial element · Juveniles · Root-knot nematode · Phenols

Extended author information available on the last page of the article
Key message

- *Lactuca sativa* is a crop susceptible to the attack of root-knot nematode.
- Silicon strengthens the defense system of this plant against *M. incognita*.
- Silicon increases tissue firmness, acting as a physical barrier in the control of *M. incognita*.
- Silicon supply decreases the reproduction rate of *M. incognita*.
- Silicon is a sustainable alternative for the control of root-knot nematode since its use can reduce nematicide applications.

Introduction

Lettuce (*Lactuca sativa* L.) is among the most popular leafy vegetables in the world. It has high economic importance (Mou 2012) and health benefits, as it is a source of vitamins and antioxidant compounds (Aksakal et al. 2017). However, most lettuce cultivars show a certain degree of susceptibility to nematodes, especially the root-knot nematode *Meloidogyne incognita*, which decreases 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 (Ornart 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, restriction in the effective control of the nematode population and its infections has serious consequences for sustainable agriculture (Sato et al. 2019). Noteworthy, chemical control is effective but not sustainable.

This requires further research to evaluate new ways of controlling nematodes. The most promising method in this context is the use of silicon. This element can provide an integrated environmentally friendly strategy as an alternative to the extensive use of pesticides (Faig et al. 2018), especially for short cycle crops such as lettuce, thus reducing risks to human health, however, there are no reports for lettuce. Research has demonstrated beneficial effects of silicon on the control of nematodes in several crops, including beet (Khan and Siddiqui 2020), rice (Zhan et al. 2018), coffee (Silva et al. 2010), banana (Oliveira et al. 2012), sugarcane (Guimaraes et al. 2010), and oats (Asgari et al. 2018).

Plants absorb silicon in the form of monosilicic acid [Si(OH)₄] (Liang et al. 2005). Transporters Lsi1 and Lsi6 belonging to the aquaporin family are primarily involved in the absorption of this element in root and shoot tissues (Mitani et al. 2011). Silicon is transported by transpiratory current and polymerized as water is lost by evapotranspiration. The increase in Si concentration induces condensation and polymerization by forming amorphous silica [SiO₂·nH₂O], also referred to as opal crystals, silica gel, or phytoliths (Richmond and Sussman 2003), which are deposited, especially in the cell wall and intercellular spaces. Nonetheless lettuce plants are not considered element- accumulators, having higher root than shoot concentrations of Si.

The mechanisms by which Si protects plants against nematodes have not been fully elucidated. Some studies indicate that the element accumulates in its polymerized form in the cell wall and extracellular spaces, forming a physical barrier that prevents penetration, feeding, and root parasitism (Dugui-Es et al. 2010; Silva et al. 2010; Asgari et al. 2018; Khan and Siddiqui 2020). Other studies propose an increase in plant resistance due to the role of Si in favoring both the production of phenolic compounds, as observed in rice plants (Rodrigues et al. 2005); and the concentration of ascorbic acid, as observed in asparagus and kale plants (Souza et al. 2019a, b). These compounds, often applied synthetically in an exogenous way (Maareg et al. 2014; Oliveira et al. 2019), have a toxic effect and can reduce nematode populations in the plant. However, it is still necessary to know whether the natural increase in these compounds, promoted by Si in the plant, is sufficient 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*.

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 plant defenses. If the hypothesis of this study is correct, it will provide first knowledge on the effectiveness of the Si-induced chemical mechanism 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 the effect of Si supply on increasing nonenzymatic compounds such as phenolic and ascorbic acid, and on physiological variables such as chlorophyll content and quantum efficiency of photosystem II, addressing the decrease both in *M. incognita* population and in oxidative stress in lettuce growth and production.
Materials and methods

Experimental site

Two experiments were carried out with lettuce cultivar Vanda, under greenhouse conditions, between March and May 2020. The experiments were established with a one-week difference so as to observe repeatability. The experimental design comprised a 3 × 2 factorial scheme: control (without inoculation of M. incognita), 6000, and 12,000 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.

The silicon source was potassium silicate (128 g L⁻¹ of Si; 126 g L⁻¹ of K₂O, pH 12), which was supplied along with the nutrient solution. Potassium chloride was used for potassium stabilization in the nutrient solution without Si between treatments.

Growth conditions

Lettuce seeds were sown in Styrofoam trays containing inert substrate (sand, washed with a 1 mol L⁻¹ HCl solution), being subsequently washed with deionized water so as to remove the excess of HCl. 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.

After transplanting, the plants were irrigated with a 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% of that concentration 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. Silicon was supplied after transplanting.

During the experimental period, the relative air humidity varied largely (69.4 ± 13.5%), maximum temperature was 34.5 ± 7.2 °C, and minimum temperature was 16.4 ± 5.7 °C (supplementary material).

Nematode inoculum

Two days after transplanting (DAT), pots were inoculated with M. incognita in both experiments. 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 (Esbenshade and Triantaphyllou 1990), using a traditional BIO-RAD Mini Protean II vertical electrophoresis system.

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 lettuce seedlings, 5 mL of the suspension was inoculated, equivalent to 6000 and 12,000 eggs and J2 of M. incognita per seedling. This corresponds to a high level infestation, capable of causing economic damage to susceptible cultivars.

To evaluate inoculum viability, a standard experiment was conducted simultaneously to the main experiment. The standard experiment included the use of eight pots with Abelmoschus esculentus under the same experimental conditions of the main experiment (substrate, fertigation frequency, temperature, and relative humidity). In addition, the nutrient solution was supplied at higher concentrations for lettuce plants during the experimental period. Root infection was subsequently evaluated using the same procedures as the main experiment.

Analysis

Silicon content

After weighing shoots and roots, the dry material was ground in a Wiley mill. To determine the Si content, 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 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 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, multiplying dry mass and element contents, and it was expressed as mg per plant (Siddiqi and Glass 1981).
**Electrolyte leakage index**

Five leaf disks were collected from the first fully developed leaf at 41 DAT and 55 DAT in the first and second experiments, respectively. The disks 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 min 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 Dionisio-Sese and Tobita (1998).

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

**Total phenols**

For total phenols, extraction and reading followed the methodology proposed by Singleton and Rossi (1965), at 41 DAT and 55 DAT in the first and second experiments, respectively. Hence, 0.1 g of fresh leaves (first fully developed leaves) were collected and subsequently placed in 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 h. 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 Foln–Cio-calteau, and the solution was allowed to rest for 3 min. After that, 1.5 mL of 20% sodium carbonate solution was added and left to react for 2 h. Finally, absorbance was read in a spectrophotometer at 765 nm. Control samples were elaborated following the same 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. Gallic acid in concentrations of 24.2 to 462 mg L−1 was used as the standard to generate the calibration curve.

**Ascorbic acid**

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. Ascorbic acid 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 FW (fresh weight) according to AOAC methodology (1980), at 42 DAT and 56 DAT in the first and second experiments, respectively.

**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, respectively.

**Photosystem II efficiency**

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

**Leaf area, fresh and dry matter, and root dry matter**

Leaf area was measured after plant sample collection, at 43 DAT and 57 DAT in the first and second experiments, respectively, using a leaf-area meter (L-3100, Li-Cor, USA). The samples were subsequently 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. The material was then packed in paper bags and dried in a forced air circulation oven at 65 ± 5 °C until reaching constant shoot dry matter and constant root dry matter.

**Estimation of nematode population number of galls, eggs, adults, and reproduction factor**

The number of eggs and galls on lettuce roots was manually and visually counted. The determination of the number of eggs and different stage specimens of *M. incognita* followed the extraction technique described by Hussey and Barker (1973), considering the methods of Coolen and D’Herde (1972). Afterward, 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 ratio between the final and initial nematode population (RF = Pf/Pi).
**Statistical analysis**

The data from both experiments were submitted to a two-way analysis of variance (ANOVA) after checking the homogeneity of the variances. The Shapiro–Wilk W test was used to test the normality of the data. Factor analysis was used to test the main effects of three levels of nematode population (POP): control (without inoculation of *M. incognita*), 6000, and 12,000 eggs and second stage juveniles (J2) of *M. incognita*; two levels of silicon (Si): presence or absence of Si (2 mM); and its interactions (POP × Si).

The independent variable was nematode population (POP), and silicon (Si) concentrations were the dependent variables. Mean values were compared using the Tukey test with a significance level of $p < 0.05$. All statistical analyses were conducted using the statistical software SAS 298 Version 9.1. It was not necessary to transform the data to meet the statistical model.

A study was carried out to measure the degree of relationships between the dependent variables using Pearson correlation coefficients ($r$) with a significance level of $p < 0.05$. All assumptions were tested: normality, linearity, and multicollinearity. Pearson correlation coefficients (Steel et al. 1997) were estimated between the 7 variables. The hypothesis that the Pearson correlation coefficient is equal to zero (H0: 0) was evaluated by the $t$ test. Analyses were performed using Agroestat software (Barbosa and Maldonado Júnior 2015). All figures were made in SigmaPlot (SigmaPlot 12.5; Systat Software, San Jose, CA).

**Results**

Silicon supply via nutrient solution decreased oxidative stress in both experiments by decreasing the rate of electrolyte leakage and increasing the content of nonenzymatic antioxidants such as phenols and AsA. The application also increased the shoot biomass of lettuce plants (Supplementary material).

**Silicon content**

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. 1A and B). However, in the first experiment, the control treatment had the highest root Si accumulation, not differing from the treatment with 12,000 eggs and J2 of *M. incognita*. Noteworthy, in the second experiment, root Si accumulation was higher in the control treatment (Fig. 1C, D). In the absence of Si, nematode population did not affect the accumulation of this element in plants of the two experiments.

In both experiments, Si application increased the accumulation of this element in lettuce roots and shoots, regardless of nematode population (0, 6000, and 12,000 eggs and J2 of *M. incognita*) (Fig. 1A–D).

![Fig. 1 Si accumulation in the shoots (A and B) and roots (C and D) of lettuce plants (cv. Vanda) from the first and second experiments, grown in pots with sand inoculated with populations (P) of 0, 6000, and 12,000 eggs and J2 of *M. incognita* per pot, which received nutrient solution in the absence (−Si) and in the presence (+Si) of 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](image-url)
Electrolyte leakage index, phenols, and ascorbic acid

Nematode populations correlated with Si for electrolyte leakage and phenol and AsA contents in both experiments (Fig. 2). In the absence of Si, inoculation with 6000 and 12,000 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 both experiments, Si application increased phenol and AsA contents in all nematode populations under study.

Chlorophyll and Photosystem II efficiency

Nematode populations correlated with Si for total chlorophyll content (Chl *a*+*b*) and quantum efficiency of PSII of lettuce leaves (Fig. 3). In the absence or presence of Si, inoculation with 6000 and 12,000 eggs and J2 of *M. incognita* decreased leaf chlorophyll content (Chl *a*+*b*) in both experiments, except in plants from Experiment 1 that received Si (Fig. 3A, B). In both experiments, Si application increased chlorophyll content (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 12,000 eggs and J2 of *M. incognita*—decreased PSII efficiency (Fv/Fm), except in plants from Experiment 2 that did not receive Si (Fig. 3C, D). In both experiments, Si application
increased PSII efficiency (Fv/Fm) in all nematode populations under study.

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. 4). In the absence or presence of Si, inoculation with 12,000 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. 4A, 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.

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. 5A). In the absence or presence of Si, inoculation with 12,000 eggs and J2 of *M. incognita* decreased shoot dry matter in the first experiment (Fig. 5A), and did not change root dry matter in any of the experiments (Fig. 5C, D).

In both experiments, Si addition to the nutrient solution increased the shoot dry matter (Fig. 5A, B) and root dry matter of lettuce plants in all nematode populations under study (Fig. 5C, D).

Estimation of nematode population number of galls, eggs, adults, and reproduction factor

The number of galls and eggs of *M. incognita* on lettuce roots, as well as the reproduction factor of the nematodes in the roots, depend on the interaction between populations and silicon (Fig. 6).

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. 6A, 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 12,000 eggs and J2 of *M. incognita* instead of control plants increased the number of nematodes on lettuce roots and the number of eggs (Fig. 6C–F). In both experiments, Si addition decreased the number of *M. incognita* on 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 12,000 eggs and J2 of *M. incognita* increased the root reproduction factor in relation to control plants (Fig. 6G, H). In both experiments, Si application in the nutrient solution decreased the reproduction...
rates of *M. incognita* in lettuce roots in the two nematode populations under study.

**Correlation coefficients in experiments 1 and 2**

Pearson correlations for the variables Si, phenols, and AsA strongly correlated with Si supply. Nonenzymatic compounds decrease the population of nematodes, the number of eggs, and the reproduction factor (Table 1).

**Discussion**

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 that improves lignification of epidermal cells (Inanaga and Okasaka 1995) and makes the cell wall more rigid and less susceptible to parasite penetration and enzymatic degradation (Faiq et al. 2018; Khan and Siddiqui 2020).
The physical benefits of Si include decreasing *M. incognita* infection in lettuce plants and increasing antioxidant compounds. 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 thus improving plant physiology.

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. Lettuce cultivation 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). In experiments 1 and 2, silicon content 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 in the leaves since it would require at least 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 greater accumulation of this element in the roots. The present research confirms these findings since Si 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. 1A–D).

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 12,000 eggs and J2 of *M. incognita* caused stress to plants, as it increased electrolyte leakage rate due to the decrease in antioxidant compounds (phenolic compounds and ascorbic acid) in the plants of the two experiments (Fig. 2).

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). These secretions weaken the tissue structure by increasing reactive oxygen species (ROS) production and lipid peroxidation (Holbein et al. 2016). Oxidative 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. 3). This physiological damage caused by nematodes that absorb water and nutrients from the plant reduced plant growth given the decrease in leaf area, number of leaves, fresh matter (especially in Experiment 1) (Fig. 4), and shoot dry matter (in both experiments), except in plants from Experiment 2 with Si (Fig. 5).

![Fig. 5 Shoot dry matter in the first (A) and second experiment (B), root dry matter in 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 12,000 eggs and J2 of *M. incognita* per pot, which received nutrient solution in the absence (−Si) and in the presence (+Si) of 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. \(n = 8\).](image-url)
Some studies have observed the effects of Si supply on the control of *M. incognita* populations in cotton plants (Santos et al. 2021). In addition to decreasing electrolyte leakage rate in lettuce plants infested *M. incognita*, the increase in antioxidant compounds also correlated with physiological aspects. The chemical effect in the reduction in oxidative stress modulated by reactive oxygen species production and electrolyte leakage (Silva et al. 2021b, a) was due to the increased content of phenolic compounds (Fig. 2C, D) and AsA (Fig. 2E, F) in the plants. Besides acting to reduce the stress caused by nematode infestation, these compounds are essential for human health (vitamin C), with AsA standing out for its production of tissue degrading compounds (Table 1) (Rokayya et al. 2013). Some authors have reported the effect of Si on oxidative stress attenuation in rapeseed and mustard (Ashfaqe et al. 2017; Hasanuzzaman et al. 2017), on the endogenous increase in AsA in chard and cabbage and rocket plants (Silva et al. 2021b, a) and in chard and kale plants (Souza et al. 2019a, b), on the increase in phenolic compounds in wheat, cabbage, and rocket plants (Ma et al. 2016; Silva et al. 2021b, a), and on the increase in antioxidant compounds such as phenols in the plants (Shahnaz et al. 2011; Hajiboland et al. 2018). 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 is noteworthy that the present study analyzed the relationships of these compounds with nematicidal action only in synthesized products (Table 1). Studies report on 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). Other studies report 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, silicon supply increased AsA content in infested and non-infested plants. In addition to decreasing the rate of infestation and inhibiting invasion, Si decreases the reproductive factor of nematodes (Table 1) and can transform susceptible plants into tolerant ones. Silicon then becomes an efficient and more environmentally friendly alternative in the control of M. incognita since its supplementation can reduce the use of nematicides (Santos et al. 2021).

Moreover, Si increased chlorophyll \((a + b)\) content and chlorophyll fluorescence efficiency (Fig. 3), indicating attenuation of oxidative stress in lettuce plants from the increase in photosynthetic efficiency. Khan and Siddiqui (2020) reported the effect of Si on the increase in both the 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. 4G, H) from the increase in leaf area (Fig. 4A, B), number of leaves (Fig. 4C, D), and fresh (Fig. 4E, F) and dry matter of shoots and roots (Fig. 5A–D) of lettuce plants from the two experiments.

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). The experiments showed that increased parasite inoculation in plants with or without Si increased the number of galls and different stage specimens of M. incognita on lettuce roots, also increasing the number of eggs and the reproduction factor (Fig. 6). This indicates that lettuce plants are susceptible to M. incognita, a fact widely reported in the literature (Franchin Sgorlon et al., 2018; Souza et al. 2019a, b).

The increase in Si uptake by lettuce plants was sufficient to decrease the number of M. incognita galls on 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 on lettuce roots, also increasing the number of eggs and the reproduction factor (Fig. 6). This indicates that lettuce plants are susceptible to M. incognita, a fact widely reported in the literature (Franchin Sgorlon et al., 2018; Souza et al. 2019a, b).

The research proposes Si supply at a concentration of 2 mM for lettuce cultivation as an additional alternative

### Table 1

Pearson simple correlation coefficients in experiments 1 and 2, between the characters root Si accumulation; nonenzymatic antioxidants (phenols, and ascorbic acid (AsA)); number of galls, J2—second stage juveniles, J3 and J4—third and fourth stage juveniles, MF—mature female, and eggs; and M. incognita reproduction factor in the roots

|                     | Root Si content | Phenols | AsA   | M. incognita galls | M. incognita J2, J3J4, MF | M. incognita eggs on roots | Reproduction factor in the roots |
|---------------------|-----------------|---------|-------|-------------------|---------------------------|----------------------------|----------------------------------|
| **Experiment 1**    |                 |         |       |                   |                           |                            |                                  |
| Phenols             | 0.58**          | –       | –     | –                 | –                         | –                          | –                                |
| AsA                 | 0.61**          | 0.86**  | –     | –                 | –                         | –                          | –                                |
| M. incognita galls  | –0.17NS         | –0.68** | –0.73** | –                | –                         | –                          | –                                |
| M. incognita J2, J4, J2, MF | –0.47**      | –0.67** | –0.69** | 0.83**            | –                         | –                          | –                                |
| M. incognita eggs on roots | –0.34*       | –0.61** | –0.62** | 0.83**            | 0.92**                    | –                          | –                                |
| Reproduction factor in the roots | –0.50**      | –0.63** | –0.59** | 0.66**            | 0.87**                    | 0.76**                     | –                                |
| **Experiment 2**    |                 |         |       |                   |                           |                            |                                  |
| Phenols             | 0.65**          | –       | –     | –                 | –                         | –                          | –                                |
| AsA                 | 0.58**          | 0.87**  | –     | –                 | –                         | –                          | –                                |
| M. incognita galls  | –0.40**         | –0.74** | –0.74** | –                | –                         | –                          | –                                |
| M. incognita J2, J4, J2, MF | –0.54**      | –0.70** | –0.74** | 0.85**            | –                         | –                          | –                                |
| M. incognita eggs on roots | –0.39*       | –0.70** | –0.67** | 0.85**            | 0.84**                    | –                          | –                                |
| Reproduction factor in the roots | –0.53**      | –0.75** | –0.80** | 0.74**            | 0.91**                    | 0.72**                     | –                                |

** and *: significant at 1 and 5% probability, respectively, by the t test
NS: Not significant by the t test
for sustainable control of *M. incognita* since it induces the defense mechanisms of plants.

**Conclusion**

The use of silicon in lettuce cultivation 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 chemical action from the increase in phenolic compounds and ascorbic acid in plant tissues, improving plant physiology.

**Author contributions**

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

**Supplementary Information** The online version contains supplementary material available at https://doi.org/10.1007/s10340-021-01470-4.

**Acknowledgements** The Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Brazil, Code 001, and Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) process 2020/00195-0, for the scholarship awarded to the first author.

**Declaration**

**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.

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