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ORIGINAL ARTICLE

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R. F. Barreto[a], R. M. Prado[a], A. J. F. Leal[b], M. J. B. Troleis[b], G. B. Silva Junior[c], C. C. Monteiro[d], L. C. N. Santos[a] and R. F. Carvalho[d]

[a]Departamento de Solos e Adubos, Universidade Estadual Paulista Júlio de Mesquita Filho, Jaboticabal, Brazil; [b]Universidade Federal de Mato Grosso do Sul, Chapadão do Sul, Brazil; [c]Departamento de fruticultura, Universidade Federal do Piauí, Teresina, Brazil; [d]Departamento de Biologia Aplicada à Agropecuária, Universidade Estadual Paulista Júlio de Mesquita Filho, Jaboticabal, Brazil

ABSTRACT

Ammonium toxicity in hydroponically grown crops can affect tomato development. However, it has been shown that the silicon (Si) attenuates ammonium toxicity in plants depending on the plant species, the stage of development and the ammonium concentration in the nutrient solution. Thus, in order to investigate how Si attenuates stress caused by ammonium in tomato, a study was carried out involving plants cultivated up to 40 days after seed germination using nutrient solutions containing ammonium concentrations (1, 2, 4, 6 and 8 mmol L^{-1}), in the absence or presence of Si (1 mmol L^{-1}). The accumulation and efficiency of nitrogen and Si use, as well as the concentrations of chlorophyll, carotenoids, malondialdehyde, hydrogen peroxide and growth parameters was assessed. At a concentration of 1 mmol L^{-1} ammonium, Si increases the accumulation of nitrogen and Si, the nitrogen use efficiency, the root area and dry biomass of the shoot. At concentrations of 1 and 2 mmol L^{-1} ammonium, Si increases the leaf area and root dry biomass, and in higher concentrations, there was no effect of Si after the supply of ammonium. It was observed that the addition of Si mitigates ammonium toxicity by 1 mmol L^{-1} ammonium, and we can recommend its use in the nutrient solution (Si = 1 mmol L^{-1}) to grow tomato cropsthat employs ammonium concentration of 1 mmol L^{-1}.

Introduction

To alleviate ammonium toxicity in plants, the use of beneficial elements such as silicon (Si) is recommended. Its effect is related to the mitigation of stresses (Liang et al. 2007; Bybordi 2010) by increasing the activity of antioxidant enzymes (Gao et al. 2014), the physical integrity of the membranes (Kochanová et al. 2014) and the photosynthetic activity (Vaculíková et al. 2014), resulting in increased plant growth. The supply of Si to tomato plants grown under salt stress can recover the content of total chlorophyll and carotenoids (Muneer et al. 2014; Li et al. 2015) and, thereby, increase the photosynthetic efficiency of the plant and mitigate the symptoms of toxicity, compared to plants cultivated in the absence of such a beneficial element (Haghighi & Pessarakli 2013).

However, there is a lack of research on the effect of Si in the mitigation of toxicity by excess of NH_4^+ to the tomato crop. In this light, there is a possibility that Si can mitigate NH_4^+ toxicity in tomato depending on the concentration of NH_4^+ in the nutrient solution.

Thus, the objective was to evaluate the relationship between the Si and the toxicity of NH_4^+ in the nutrition, physiology and production of dry biomass of the tomato (cv Moneymaker) grown hydroponically.

Materials and methods

Plant material and growth conditions

Tomato seeds (cv Moneymaker) were sown in trays containing 1 : 1 (v:v) of pine bark plus coconut fiber and expanded vermiculite, in a greenhouse with misting activated for 1 min every hour.

At nine days after emergence (DAE), plants were transferred into polyethylene vessels containing sponges to hold the plants, kept under ambient temperature of 25°C, 12 h photoperiod and light intensity of 250–400 µmol m^{-2} s^{-1}. Vessels of 9.5 cm top width, 8 cm bottom width and 3 cm in height, contained 180 mL aerated solution (Hoagland & Arnon 1950), using Fe-EDDHA as the iron source.
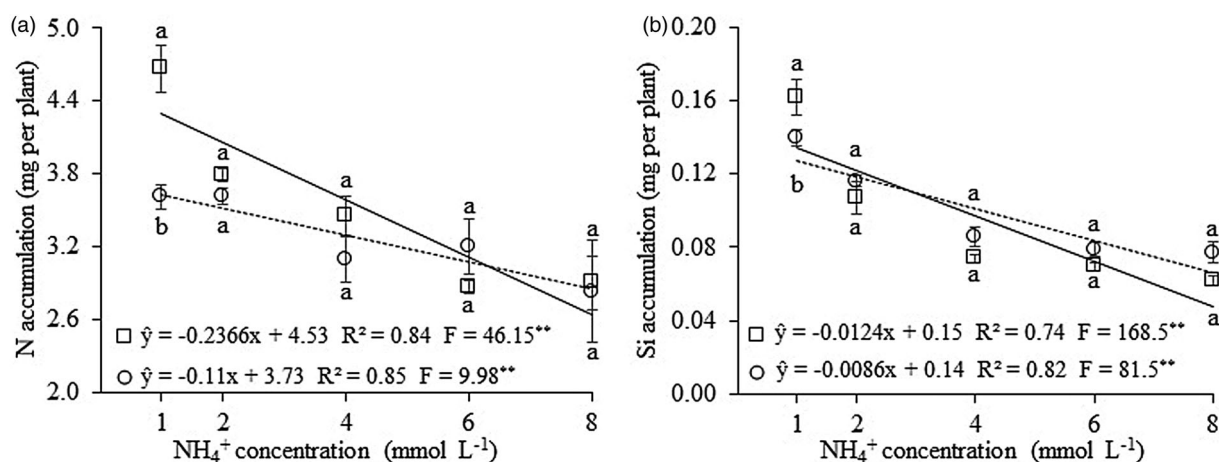
CONTACT R. F. Barreto ✉ rafael.fb@outlook.com ▣ Departamento de Solos e Adubos, Universidade Estadual Paulista Júlio de Mesquita Filho, Via de Acesso Prof. Paulo Donato Castellane s/n, CEP 14884900, Jaboticabal, Brazil

Figure 1. Effects of NH_4 concentrations, in the presence (□) and in the absence of Si (○) on N accumulation (a) and Si accumulation (b) in the shoots of tomato, at 40 DAE. Different letters in the same concentration of NH_4^+ indicate significant differences by the *F*-test ($P \leq .05$). Standard error regards the average of four replicates.

Initially, a nutrient solution with 25% ionic strength was used. Yet at 16 DAE, the ionic strength of the solution was raised to 50% and maintained until the end of the experiment. Distilled water was replaced every three days. Each day, the pH was adjusted (5.7 ± 0.3) using HCl 1.0 mol L^{-1} solution and KOH 1.0 mol L^{-1}.

Treatments and experimental design

A completely randomized design was adopted, with four replicates, in a 2×5 factorial scheme, corresponding to the absence and presence of Si (1 mmol L^{-1}) and five ammonium concentrations (1, 2, 4, 6 and 8 mmol L^{-1}). The Si was supplied as monosilicic acid, at 30 DAE, and as the N source NH_4^+ or ammonium chloride was applied at 24 DAE. The replicate consisted of a pot with four plants.

Analyses

After the end of the experimental period (10 and 16 days after the supply of Si and NH_4^+, respectively), plants were collected for determination of parameters: lipid peroxidation, using the test of 2-thiobarbituric acid (TBA). As end products of the lipid peroxidation process, malondialdehyde (MDA) levels were determined photometrically by readings at 535 and 600 nm (Heath & Packer 1968); the content of hydrogen peroxide (H_2O_2) was determined according to Alexieva et al. (2001). Plant tissues were homogenized in 0.1% (m/v) trichloroacetic acid (TCA). The homogenate was centrifuged at $12,100 \times g$, 15 min and 4°C, and 200 μL of the supernatant was added to 200 μL of 100 mM potassium phosphate buffer (pH 7.0) and 800 μL of 1

M KI. The absorbance was read at 390 nm. H_2O_2 content for all samples was determined using H_2O_2 as a standard. Further measurements were conducted as follows: content of photosynthetic pigments, with readings at 661.6 nm for chlorophyll *a*, 644.8 nm for chlorophyll *b* and 470 nm for carotenoids (Lichtenthaler 1987); leaf area was determined using an area meter (LI-3100 C, Li-COR) and root systems were scanned to produce digital images in uncompressed tagged image file (TIF) format by a scanner (ScanJet 4c, Hewlett-Packard Co.). An image analysis software (Delta-T Devices) was used to analyze the images and to estimate the root area; dry biomass of root and shoot, from the drying of the plant material in forced air circulation oven ($65 \pm 5°C$) until constant weight; and chemical analyses of N (Bataglia et al. 1983) and Si (Kraska & Breitenbeck 2010) contents. From the contents of these elements and of the dry biomass, the N and Si accumulation, the N use efficiency (NUE) and the Si use efficiency (SiUE) in the shoots were calculated (Fageria & Baligar 2005).

Data were subjected to analysis of variance, followed by the comparison of means of the absence and presence of Si by the *F*-test ($P \leq .05$), and polynomial regression analysis for the NH_4^+ concentrations, using the software Sisvar (Ferreira 2011).

Results

Both in the presence and absence of Si in the nutrient solution, the increase in NH_4^+ concentrations led to a linear decrease in the accumulation of N (Figure 1(a)), Si (Figure 1(b)) and NUE (Figure 2(a)).

The SiUE decreased with increasing concentrations of NH_4^+ (Figure 2(b)) in the culture medium. However,
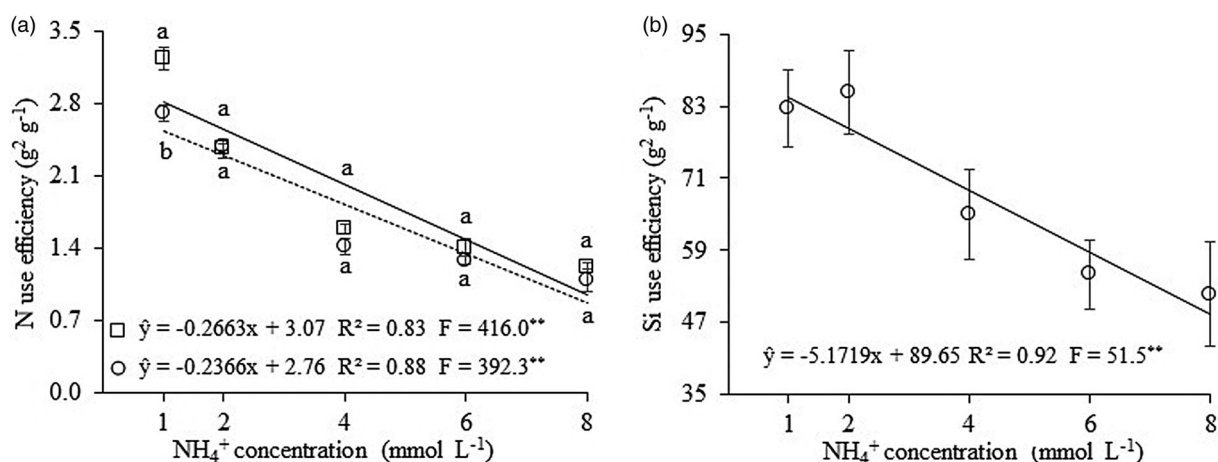
Figure 2. Effects of NH_4 concentrations, in the presence ($\square$) and in the absence of Si ($\bigcirc$) on NUE (a) in the shoots of tomato, at 40 DAE. Different letters in the same concentration of NH_4^+ indicate significant differences by the F-test ($P \leq .05$). Mean standard error regarding four replicates. Effects of NH_4^+ concentrations on SiUE (b) in the shoots of tomato, 40 DAE. ($\bigcirc$) Means of the concentrations of 0 and 1 mmol L^{-1} Si. Standard error regards the average of eight replicates.

the enrichment of the nutrient solution with Si (1 mmol L^{-1}) resulted in the highest use efficiency of that element. Thus, at the concentration of 1 mmol L^{-1} NH_4^+, the provision of Si enabled higher accumulation of N (Figure 1(a)), Si (Figure 1(b)) and increased NUE (Figure 2(a)).

Regardless of the concentration of NH_4^+, the MDA content was lower with the supply of Si in the nutrient solution. Nonetheless, there was an increase in MDA content in the tomato leaves, with quadratic adjustment depending on NH_4^+ concentrations, reaching the point of maximum accumulation at the concentration of 5.43 mmol L^{-1} NH_4^+ (Figure 3(a)). The same effect was observed for the H_2O_2 content, with maximum accumulation at the concentration of 4.56 mmol L^{-1} NH_4^+ (Figure 3(b)).

The supply of Si increased the content of total chlorophyll and carotenoids. In contrast, the increase in NH_4^+ concentrations reduced the content of total chlorophyll (Figure 4(a)) and carotenoids (Figure 4(b)).

Root area and leaf area decreased with the increase in NH_4^+ concentration (Figure 5(a) and (b)), as well as the dry biomass of root and shoot (Figure 6(a) and (b)), regardless of the enrichment of the nutrient solution with Si. However, at a concentration of 1 mmol L^{-1} NH_4^+, the presence of Si increased the root area and the dry biomass of shoots (Figures 5(a) and 6(b)). As for the concentrations of 1 and 2 mmol L^{-1} NH_4^+, Si increased the leaf area and the dry biomass of roots (Figures 5(b) and 6(a)).
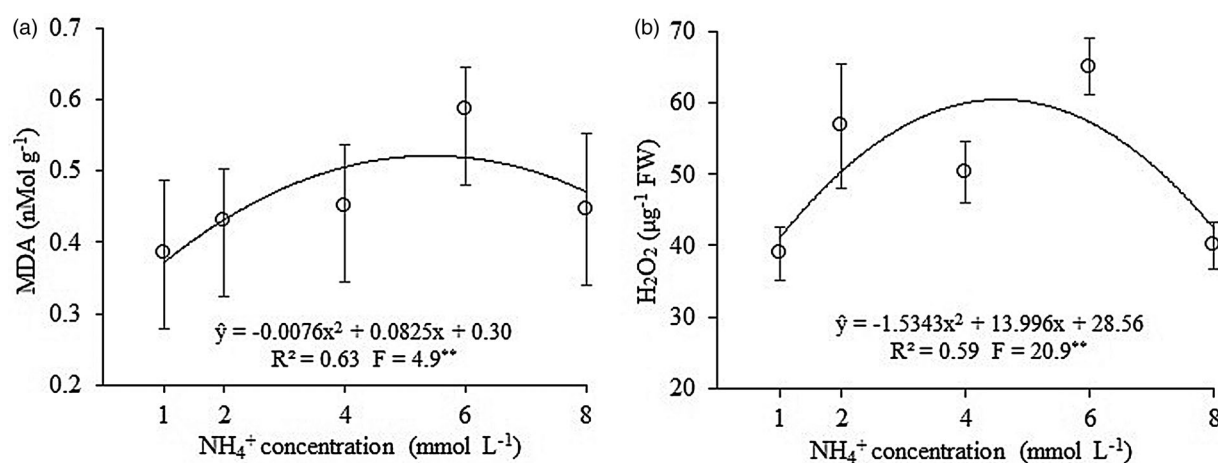

Figure 3. Effects of NH_4^+ concentrations on content of MDA (a) and H_2O_2 (b) in the tomato leaves, at 40 DAE. ($\bigcirc$) Means of the concentrations of 0 and 1 mmol L^{-1} Si. Standard error regards the average of eight replicates.
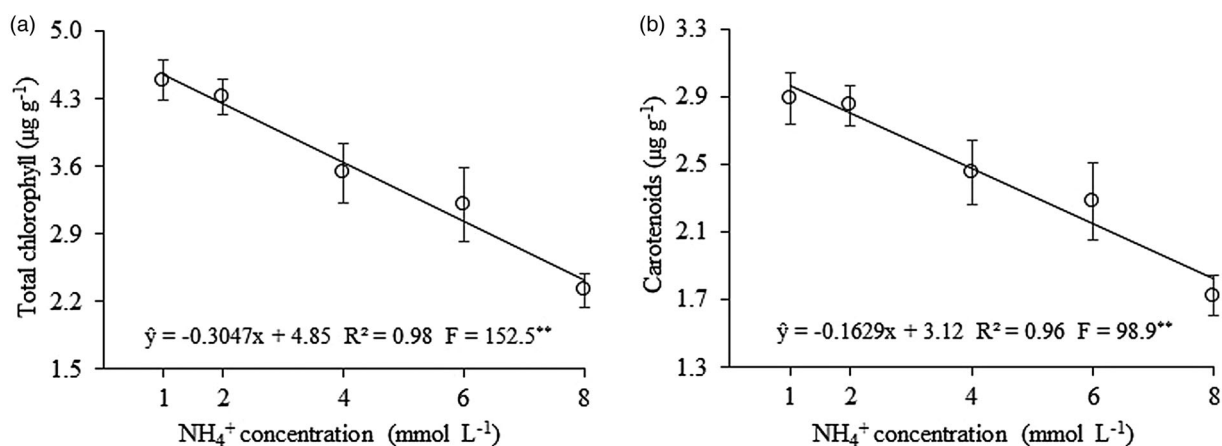
Figure 4. Effects of NH_4^+ concentrations on content of total chlorophyll (a) and carotenoids (b) in the tomato leaves, at 40 DAE. (◯) Means of the concentrations of 0 and 1 mmol L^{-1} Si. Standard error regards the average of eight replicates.

Discussion

The presence of Si raised N accumulation (Figure 1(a)), Si accumulation (Figure 1(b)) and NUE (Figure 2(a)). This observation supports the evidence that the beneficial effects of Si, such as increased accumulation of nutrients and increased production of dry biomass per NH_4^+ in toxicity conditions, are depending on the concentration of this cation in the nutrient solution (Campos et al. 2015).

Accordingly, despite the higher SiUE with the supply of Si, there was a reduction in N accumulation (Figure 1(a)), Si accumulation (Figure 1(b)), NUE (Figure 2(a)) and SiUE (Figure 2(b)) at the concentration of 1 mmol L^{-1} NH_4^+ or higher, independent of the supply of Si due to the toxicity caused by this cation in high concentrations in plant tissue (Bittsánszky et al. 2015).

Thus, Borgognone et al. (2013) observed a decrease in the accumulation of calcium in tomato grown (cv Moneymaker) in the nutrient solution containing high concentrations of NH_4^+ (23 mmol L^{-1}). In contrast, Ghanem et al. (2010) observed no reduction in the accumulation of N in the shoots in nutrient with 6.5 mmol L^{-1} N, with low concentration of NH_4^+ in the nutrient solution (1.5 mmol L^{-1}), caused no reduction with respect to the accumulation of N in the shoots. Therefore, in this genetic material from tomato, low concentrations of NH_4^+ do not decrease the concentration of N in comparison with high concentrations, as seen in this experiment (Figure 1(a)).

The increase in total chlorophyll and carotenoid contents in the presence of Si indicates that this beneficial element acts in retaining photosynthetic pigments under conditions of mineral toxicity. A similar response was observed for *Oryza sativa*, grown in zinc deficiency and *Zea mays* under arsenic toxicity,

in which the supply of Si in the nutrient solution increased the total chlorophyll content, associated with stress relief resulting in the protection of the chloroplast structure, exerted by Si (Song et al. 2014; Silva et al. 2015).

Notwithstanding, the content of total chlorophyll (Figure 4(a)) and carotenoids (Figure 4(b)) followed the same pattern as SiUE (Figure 2(b)), that is, decreased from the concentration of 1 mmol L^{-1} NH_4^+. However, Horchani et al. (2010) indicated that, for tomato (cv Rio Grande), there was an increase in the total chlorophyll content in the highest NH_4^+ concentration studied (10 mmol L^{-1}) and greater accumulation of carotenoids in the supplying of 7.5 mmol L^{-1} NH_4^+, while maintaining the accumulation of N in the leaves, indicating a possible interaction between tomato genotypes and tolerance to higher concentrations of NH_4^+. The decrease in total chlorophyll content with increase in NH_4^+ concentrations in nutrient solution was verified in *Spinacia oleracea* plants, when N was supplied as NH_4^+, and the increase in this cation decreased N accumulation in the shoots, reflecting the lower concentration of chlorophyll (Xing et al. 2015), as occurred in this experiment (Figure 4(a)), because N is in the chloroplasts of leaves, being a constituent of the chlorophyll molecule (Hoertensteiner 2006).

As the MDA content, which is a product of lipid peroxidation caused by H_2O_2 is suitable to indicate stress damage in plants (Dewir et al. 2006), the increase in these compounds affects the cell components reducing the total content of chlorophyll, carotenoids (Figure 4(a) and (b)), lipid and protein and, depending on the concentration, make them unfunctional (Gill & Tuteja 2010). This fact constitutes a possible explanation for the reduction in MDA and H_2O_2
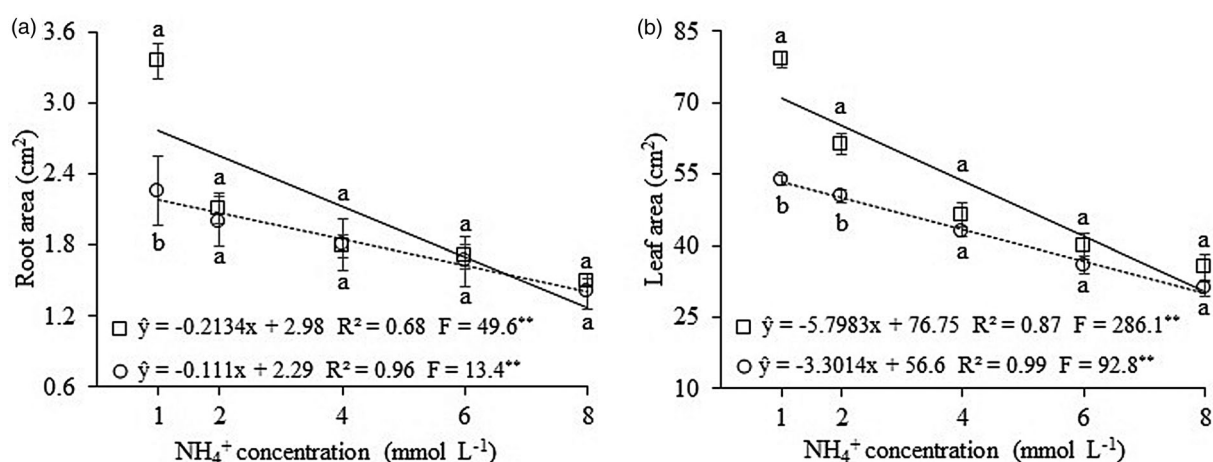
Figure 5. Effects of NH_4^+ concentrations, in the presence ($\square$) and in the absence of Si ($\bigcirc$) on root area (a) and leaf area (b) of tomato plants, at 40 DAE. Different letters in the same concentration of NH_4^+ indicate significant differences by the *F*-test ($P \leq .05$). Standard error regards the average of four replicates.

accumulation in the tomato leaves, from the points of maximum accumulation (Figure 3(a) and (b)). Similarly, Nasraoui-Hajaji and Gouia (2014) observed inhibition of the photosynthetic activity regarding at a supply of 5 mmol L^{-1} NH_4^+ in tomato. Although the reduction in the MDA content observed in the presence of Si indicates that the H_2O_2 content is lower in the presence of the beneficial element, this event was independent of the supply of Si. It shows that the biochemical line is not obviates, mainly in oxidative stress, as mentioned by Monteiro et al. (2011).

The response of the growth variables of tomato such as root and leaf area (Figure 5(a) and (b)), and dry biomass of root and shoot (Figure 6(a) and (b)) to the supply of Si and concentrations of NH_4^+ followed the accumulation behavior of N and Si

(Figure 1(a) and (b)). Hence, the supply of 1 mmol L^{-1} Si after the concentration of 1 mmol L^{-1} NH_4^+, where tomato showed less content of MDA and H_2O_2 (Figure 3(a) and (b)), that is, less stress, allowed greater plant growth. It was observed that the addition of Si mitigates ammonium toxicity by 1 mmol L^{-1} ammonium, and we can recommend its use in the nutrient solution (Si = 1 mmol L^{-1}) to grow tomato crops, which employ ammonium concentration of 1 mmol L^{-1}.
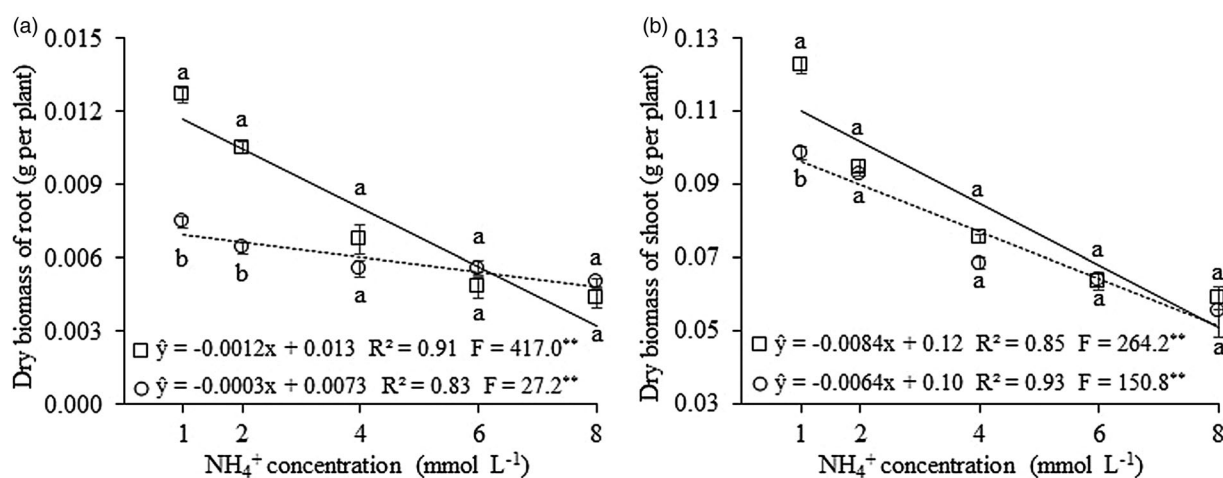
Acknowledgements

Figure 6. Effects of NH_4^+ concentrations, in the presence ($\square$) and in the absence of Si ($\bigcirc$) on dry biomass of root (a) and dry biomass of shoots (b) of tomato plants, at 40 DAE. Different letters in the same concentration of NH_4^+ indicate significant differences by the *F*-test ($P \leq .05$). Standard error regards the average of four replicates.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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