

Silicon mitigates ammonium toxicity in yellow passionfruit seedlings

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Received: 30 October 2018; Accepted: 24 January 2019; doi:10.4067/S0718-58392019000300425

ABSTRACT

Ammonium (NH_4^+) toxicity in yellow passionfruit (*Passiflora edulis* Sims f. *flavicarpa* O. Deg.) may be mitigated by Si application. This study aimed to evaluate the interaction effect of Si and high level of NH_4^+ on yellow passionfruit seedlings nutrition, physiology, growth, and DM production. Pots were filled with pinus bark and nutrition solution was applied. Treatments were arranged in a completely randomized design, with five replicates, in a 2×2 factorial scheme: two ratios of NH_4^+ and nitrate, NO_3^- (40/60%, without high level of NH_4^+ ; and 75/25%, with high level of NH_4^+) at N concentration of 13 mmol N L^{-1} , in the absence and presence of Si (2.0 mmol L^{-1}). Sixty days after seedling transplant it was evaluated: N, Si, K, Ca and Mg root and shoots accumulation, leaf green color index (GCI), electrolyte leakage index (ELI), intracellular CO_2 concentration (C_i), transpiration rate (T_r), stomatal conductance (g_s), net photosynthesis rate (P_n), stem diameter, leaf area, root length, N use efficiency (NUE) and root and shoot DM content. Plants cultivated with Si had 19.1% and 16.3% lower T_r and g_s , respectively, regardless NH_4^+ concentration. Moreover, C_i and P_n were 13.2% and 16.4%, respectively, higher in plants that received Si. Plants cultivated under high NH_4^+ concentrations, with Si application had 17% bigger stem diameter and 15.4% bigger root length than plants without Si application. Si application in yellow passionfruit seedlings cultivated with high level of NH_4^+ increased accumulation and NUE, root length, root DM, and GCI. In addition, Si application reduced ELI, which resulted in higher stem diameter. These results prove that Si application mitigates NH_4^+ toxicity in yellow passionfruit seedlings.

Key words: Abiotic stress, beneficial element, NH_4^+ , nitrogen, *Passiflora edulis*, toxicity.

INTRODUCTION

The N form taken up by plants affects its growth and development; hence, nitrate and ammonium proportions in nutrient solution is an important issue. Nitrogen supply through ammonium (NH_4^+) form requires lower energy consumption by the plants. Different from nitrate (NO_3^-), ammonium is directly incorporated into the C chain without the need of an enzymatic reduction, which is energy dependent (Bittsánszky et al., 2015). However, it has been observed that NH_4^+ supply in excess causes toxicity symptoms in most plant species (Li et al., 2013).

Ammonium toxicity in plants is due to an increase in reactive oxygen species, as well as a decrease on cytoplasmic pH, photosynthetic rate (Hu et al., 2015), and chlorophyll content, which affects membrane integrity (Kochanová et al., 2014). Moreover, high level of NH_4^+ competes with other cations, such as K, Ca, and Mg, during plant uptake process, reducing its accumulation; thus, plant DM production (Mendoza-Villarreal et al., 2015; Chen et al., 2016).

In order to mitigate ammonium toxic effect in plants, some management practices might be adopted, for example, the application of beneficial elements such as Si. Although Si is not considered as a plant essential nutrient, it is responsible to protect plants against abiotic stresses (Kurdali et al., 2018), including drought, salinity, heat, cold, heavy metal toxicity (Liang et al., 2007), and nutrient imbalance, for example, ammonium toxicity (Prado et al., 2017).

Silicon beneficial effect in plants under stressed conditions is due to stimulation of plant antioxidant systems, which enhances membrane physical integrity (Kochanová et al., 2014; Barreto et al., 2018), to protect plant photosynthetic apparatus (Vaculíková et al., 2014), and to enhance nutrient uptake (Chen et al., 2016; Barreto et al., 2017; Kurdali et al., 2018; Mantovani et al., 2018). Therefore, Si enhances vegetative growth through this beneficial effect.

Silicon mitigating effect in plants under high level of ammonium has been studied in *Brassica oleracea* (Barreto et al., 2017), *Zea mays* (Campos et al., 2015), *Solanum lycopersicum* (Barreto et al., 2016), and *Cucumis sativus* (Campos et al., 2016). However, the role of Si in plant nutrition and in each organ of the plant is ambiguous. Moreover, there are lack of studies regarding Si and ammonium toxicity on seedlings of fruits, especially on seedlings of yellow passionfruit. Therefore, the hypothesis of this study is that ammonium toxicity in yellow passionfruit seedlings might be mitigated by application of Si through nutrient solution.

The objective of this study was to evaluate the interaction effect of Si and high level of ammonium on yellow passionfruit seedlings nutrition, physiology, growth, and DM production.

MATERIALS AND METHODS

Experiment location and establishment

The study was conducted between May and July 2014 at the greenhouse of São Paulo State University, campus Jaboticabal (21°15'22" S, 48°18'58" W; 610 m a.s.l.), Brazil.

Yellow passionfruit (Hybrid BRS Rubi do Cerrado) seeds were sown in 53 cm³ pots filled with vermiculite. Pots were daily watered and seedlings (two pairs of fully formed leaves) were obtained 20 d after sowing. Yellow passionfruit seedlings were transferred to 1.7 L polypropylene pots filled with sterilized pine bark (grain size: 6.0 mm, density: 275.9 kg m⁻³, and water retention capacity: 1.32 kg kg⁻¹) in which the experiment was established.

The treatments (N source and Si application) were arranged in a completely randomized design, with five replicate, in a 2 × 2 factorial scheme: two ratios of NH_4^+ and NO_3^- (40/60%, low level NH_4^+ [5.2 mmol L⁻¹ NH_4^+]; and 75/25%, with high level of NH_4^+ [9.75 mmol L⁻¹ NH_4^+]), in the presence or absence of Si at 2.0 mmol L⁻¹. Nitrogen was applied at 13 mmol L⁻¹ and ammonium and nitrate ratios were determined based on previous experiment. Each experimental unit consisted of four individually grown seedlings in a polypropylene pot with a 1.7 L volume (diameter: 12 cm; bottom diameter: 10 cm; height: 20 cm).

Seedlings growth conditions

Yellow passionfruit seedlings were grown in a Hoagland and Arnon (1950) nutrient solution modified according to N and Si treatments, and with Fe-ethylendiamine di(2-hydroxy-4-methylphenylacetic) acid (EDDHMA) as Fe source (Table 1). The sources of NH_4^+ and NO_3^- were NH_4Cl (N = 26.4%) and $\text{Ca}(\text{NO}_3)_2 \cdot 5\text{H}_2\text{O}$ (N = 11.1%), respectively,

Table 1. Nutrient solution content modified according to NH_4^+ and NO_3^- ratios and Si treatments.

$\text{NH}_4^+/\text{NO}_3^-$	Si	NH_4^+	NO_3^-	P	K	Ca	Mg	S
(%)		mmol L ⁻¹						
40/60	0.0	5.2	7.8	1.0	6.0	7.8	2.0	2.0
40/60	2.0	5.2	7.8	1.0	6.0	7.8	2.0	2.0
75/25	0.0	9.8	3.2	1.0	6.0	7.8	2.0	2.0
75/25	2.0	9.8	3.2	1.0	6.0	7.8	2.0	2.0

whereas monosilicic acid ($\text{Si} = 28.5 \text{ g L}^{-1}$) was used as Si source. Treatments were balanced for Ca concentration using $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$. Further macronutrients fertilization was done using KH_2PO_4 , KCl and $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$. Micronutrients were provided through Hoagland and Arnon (1950) solution which contained: $2.86 \text{ g H}_3\text{BO}_3 \text{ L}^{-1}$; $1.81 \text{ g MnCl}_2 \cdot 4\text{H}_2\text{O L}^{-1}$; $0.10 \text{ g ZnCl}_2 \text{ L}^{-1}$; $0.04 \text{ g CuCl}_2 \text{ L}^{-1}$; $0.02 \text{ g H}_2\text{MoO}_4 \cdot \text{H}_2\text{O L}^{-1}$ and $40.0 \text{ g Fe-EDDHMA L}^{-1}$. The nutrient solution pH was maintained at 6.0 ± 0.1 .

Nutrient solutions adjusted according to the treatments (Table 1) were daily applied to the pots at 50 mL until 20 d after transplanting (DAT). From 21 to 35 DAT 100 mL solution were applied, whereas from 36 to 60 DAT the solution was applied at 150 mL. Polypropylene plates (150 mL) were placed at the base of each pot in order to collect the nutrient solution. The nutrient solution was manually returned to the pots at the end of each day. Application volumes were determined based on the plant development observed in previous experiments.

A digital thermo-hygrometer (thermo-hygrometer Instrutherm, HT-210, São Paulo; São Paulo State, Brazil) were used to collect daily air temperature and relative humidity at the greenhouse. Data were collected between August and September of 2014, from seedlings transplant to plant harvest (Figure 1).

Photosynthetic parameters

At 60 DAT, intracellular CO_2 concentration (C_i ; $\mu\text{mol mol}^{-1}$), transpiration rate (T_r ; $\text{mmol m}^{-2} \text{ s}^{-1}$), stomatal conductance (g_s ; $\mu\text{mol m}^{-2} \text{ s}^{-1}$), and net photosynthesis rate (P_n ; $\mu\text{mol m}^{-2} \text{ s}^{-1}$) were measured. At this stage, seedlings had 8-10 vigorous leaves and the first visible tendril. Evaluations were performed at the fourth fully developed leaf from the apex to the bottom, as indicated by Malavolta et al. (1997) as the diagnosis leaf of yellow passionfruit seedlings. Data were collected from two plants per pot, between 08:00 and 10:00 h, under CO_2 ambient concentration ($372 \pm 10 \text{ mol m}^{-2} \text{ s}^{-1}$), and photosynthetically active photon flux of $1200 \text{ mol m}^{-2} \text{ s}^{-1}$. An infrared gas analyzer (LI-6400; LI-COR, Lincoln, Nebraska, USA) was used.

Leaf green color and electrolyte leakage indexes

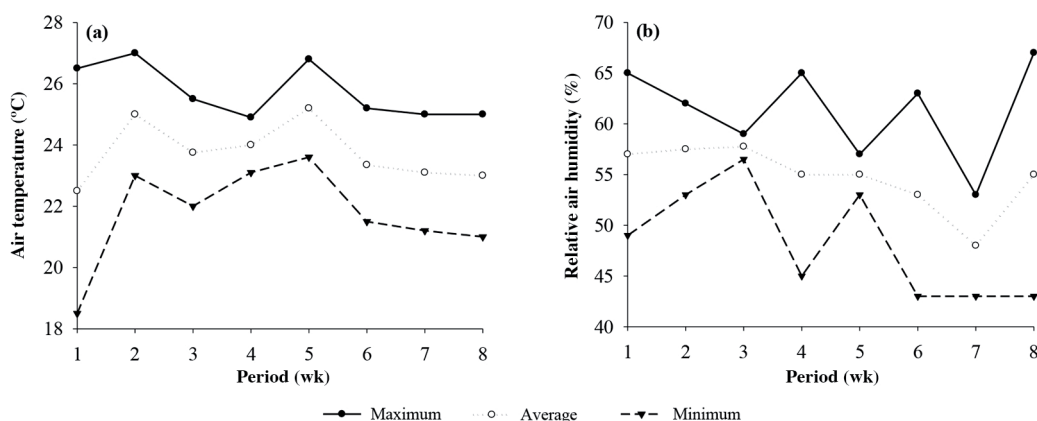
Leaf green color index was measured using a portable chlorophyll meter (CCM-200; Opti-Sciences, Hudson, New Hampshire, USA). The readings were taken between 11:00 and 12:00 h at the upper, middle and lower part of the leaves. Moreover, membrane physical integrity of leaf cells was determined based on the electrolyte leakage index. A conductivity meter was used (CD 880; Instrutherm, São Paulo, São Paulo State, Brazil) as suggested by Dionisio-Sese and Tobita (1998). Data were collected from 5 mm-cuts of fresh leaves weighting 100 mg each.

Plant parameters and nutritional composition

At 60 DAT, the stem diameter (mm) was measured 2.0 cm from the base of the plant, using a digital caliper (727-2001; Starrett, Athol, Massachusetts, USA), whereas leaf area (cm^2) was obtained through the sum of all leaves area taken in each plant using a digital meter (model L1-3000; LI-COR, Lincoln, Nebraska, USA). Root length (cm) was also measured.

Plants were harvest, washed, and separated into shoots and roots before drying it in air forced oven at $65 \pm 2 \text{ }^\circ\text{C}$. Roots and shoots dry weight were determined.

Figure 1. Air temperature (a) and relative air humidity (b) at the greenhouse during experiment.



After drying, plants were ground in a Wiley mill (Thomas Scientific, Swedesboro, New Jersey, USA) and N, K, Ca and Mg contents determined according to Bataglia et al. (1983) methodology. N content was determined by the Kjeldahl method. For the K, Ca and Mg content, 0.2 g dried samples were added in 5 mL perchloric acid and 15 mL nitric acid and incubated overnight at room temperature, then digested at 180 °C using a heating block, and diluted to a volume of 50 mL with distilled demonized water. The concentrations of K, Ca, and Mg, was determined by atomic absorption spectrometer (SpectrAA220FS, Varian, Palo Alto, California, USA). Silicon content was determined according to Kraska and Breitenbeck (2010) procedure. The Si contents of rice leaves were determined by the colorimetric molybdenum blue method. In brief, 0.3 g samples of yellow passionfruit leaves were ashed in porcelain crucibles for 3 h at 550 °C, the ash was dissolved in 1.3% hydrogen fluoride, then the Si concentrations in the solutions were measured by the colorimetric molybdenum blue method at 811 nm with a spectrophotometer (V-M5, Bel Engineering, Monza, Italy). Based on nutrient content and plant DM, root and shoot nutrient accumulation was calculated. The N use efficiency (NUE, g g⁻¹) was also calculated from root and shoot N accumulation through Fageria (1998) formula: $NUE = (\text{Root or shoot DM mass})/\text{Root or shoot N accumulation}$.

Statistical analysis

The data were analyzed through ANOVA based on F test. Mean comparison of NH₄⁺ and NO₃⁻ ratios and Si treatment were also done using Tukey's test ($P \leq 0.05$). A Pearson correlation analysis ($P \leq 0.05$) was conducted among variables. The software SisVar was used for all statistical analysis (Ferreira, 2011).

RESULTS AND DISCUSSION

Plant physiological parameters

The ANOVA of NH₄⁺ and NO₃⁻ ratios and Si application are presented in Table 2. It was observed a significant interaction effect between NH₄⁺ and NO₃⁻ ratios and Si on green color and electrolyte leakage indexes ($P \leq 0.05$) (Table 2). Under high concentration of ammonium, plants applied with Si had 44.1% higher leaf green color index than plants without Si application (Figure 2a). However, in the presence of Si, there was no increase in the leaf green color index in plants grown in nutrient solution with low ammonium concentration. Unlike in systems containing low NH₄⁺ concentration, Si uptake is enhanced in plants under high ammonium or in stressed conditions, which is elucidated by a positive correlation between Si accumulation and leaf green color index ($r = 0.80^{**}$).

Similar results were observed by authors that studied Si and NH₄⁺ toxicity interaction in other plants (Hu et al., 2015; Campos et al., 2016; Barreto et al., 2017). The toxic effect of NH₄⁺ is possibly due to increased content of reactive oxygen species (O₂⁻ and H₂O₂) in plants under high concentration of NH₄⁺, which may cause oxidative peroxidation and reduce leaf chlorophyll content (Prado et al., 2017; Barreto et al., 2018). Moreover, chlorosis and necrosis in older leaves of yellow

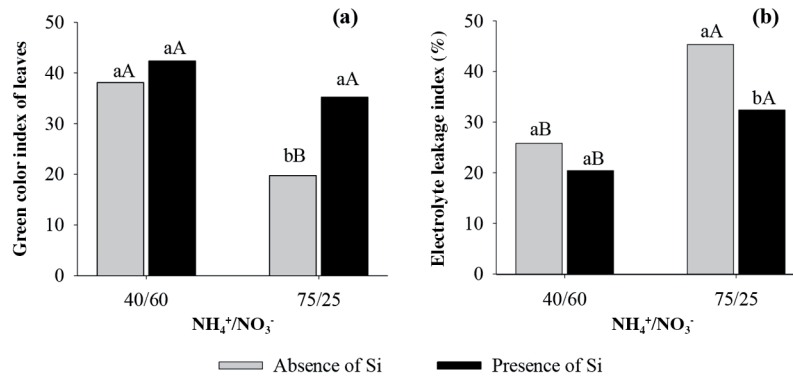
Table 2. ANOVA of leaf green color index (GCI), electrolyte leakage index (ELI), intracellular CO₂ concentration (C_i) transpiration rate (T_r), stomatal conductance (g_s), and net photosynthesis rate (P_n) of yellow passionfruit seedlings under different NH₄⁺ and NO₃⁻ ratios with and without Si application.

Treatment	GCI	ELI	C _i	T _r	g _s	P _n
	— % —		μmol mol ⁻¹	mmol m ⁻² s ⁻¹	— μmol m ⁻² s ⁻¹ —	
NH ₄ ⁺ /NO ₃ ⁻ (R)	24.6*	124.7*	3.0 ^{ns}	7.4**	1.9 ^{ns}	0.9 ^{ns}
40/60	40.6	23.1	182.9	4.0	0.092	9.8
75/25	27.5	38.9	171.3	4.6	0.098	9.3
LSD	5.6	3.0	-	0.2	-	-
Silicon (Si)	14.9**	41.9**	14.4**	48.3**	17.0**	4.0*
Absence	29.0	35.6	164.4	4.2	0.104	9.2
Presence	39.1	26.4	189.4	3.4	0.087	11.0
LSD	5.6	3.0	14.2	0.2	0.008	1.3
R×Si	7.1*	7.1*	4.4 ^{ns}	1.1 ^{ns}	0.2 ^{ns}	1.8 ^{ns}
CV, %	10.3	10.2	8.5	6.5	9.8	14.6

LSD: Least significant difference; CV: coefficient of variation; ^{ns}: nonsignificant.

*, **Significant at the 0.05 and 0.01 probability levels, respectively, according to F test.

Figure 2. Leaf green color index (a) and electrolyte leakage index (b) of yellow passionfruit seedlings under different NH_4^+ and NO_3^- ratios with or without Si application.



Means followed by different lower-case letters indicate Si significant effect within NH_4^+ and NO_3^- ratios and uppercase letters indicate NH_4^+ and NO_3^- ratios significant effect within Si concentrations, according to Tukey's test ($P \leq 0.05$).

passionfruit seedlings indicated a nutritional plant disorder under NH_4^+ toxicity. These symptoms were also reported by Campos et al. (2016) and Barreto et al. (2018) in *Cucumis sativus* and *Solanum lycopersicum*, respectively, grown in nutrient solution containing high concentration of NH_4^+ .

The electrolyte leakage index of yellow passionfruit leaves increased at high concentration of NH_4^+ , regardless Si application ($P \leq 0.05$) (Figure 2b). This resulted from the membrane damage and cell wall breakage, which increase cell electrolyte leakage (Kochanová et al., 2014). Similar effect was observed in plants of *Arabidopsis thaliana* (Helali et al., 2010), *Brassica oleracea* (Barreto et al., 2017), and *S. lycopersicum* (Barreto et al., 2018) cultivated under high NH_4^+ concentrations. In contrast, plants under high NH_4^+ concentration that received Si fertilization had lower electrolyte leakage index than plants without Si (Figure 2b). This might be explained by plant enhanced uptake of Si and Ca, as showed through the negative correlation between electrolyte leakage index and Si ($r = -0.71^{**}$) and electrolyte leakage index and Ca ($r = -0.71^{**}$). Silicon increases membranes physical integrity of plants under different abiotic stress, as noticed by Kochanová et al. (2014) in *Zea mays* and Li et al. (2012) in *Cucumis melo*. Moreover, Si connects to free monophenols, which forms a Si-phenol compound that acts avoiding accumulation of reactive oxygen species in the cell (Maksimovic et al., 2007), and reduces lipid peroxidation, which preserve cell chloroplasts and organic compounds (Hernandez-Apaolaza, 2014; Kochanová et al., 2014). In addition, Ca is an essential nutrient responsible for cell membrane integrity (Kochanová et al., 2014), which also contributes to plant lower electrolyte leakage index.

Yellow passionfruit seedlings leaf transpiration (T_r) was 13.1% higher in plants under high concentration of NH_4^+ than in plants under low concentration ($P \leq 0.01$) (Table 2). High concentration of NH_4^+ might decrease intracellular pH and cause an osmotic imbalance, which increase reactive oxygen concentration. Under oxidative stress, plants may modify its physiological activities, such as transpiration rate (Bittsánszky et al., 2015). In contrast, plants applied with Si had 19.1% and 16.3% lower T_r and g_s , respectively, regardless NH_4^+ concentration. Moreover, C_i and P_n were 13.2% ($P \leq 0.01$) and 16.4% ($P \leq 0.01$), respectively, higher in plants that received Si (Table 2). Silicon benefits plant physiological activities, especially photosynthesis, due to its deposition on the abaxial leaf surface. This deposition help plant maintain its leaves erect, which increase light interception (Feng et al., 2010), and active photosynthetic area (Kurdali et al., 2013). Silicon deposition beneath leaf epidermis forms a double-layer that affects g_s and intracellular CO_2 concentration; thus affecting photosynthetic efficiency (Kochanová et al., 2014). Higher photosynthetic efficiency and growth were reported in *Cicer arietinum* (Kurdali et al., 2013), and *Z. mays* (Vaculíková et al., 2014) plants cultivated in a system containing Si.

Plant N, Si, K, Ca, and Mg accumulation

There was a significant interaction between $\text{NH}_4^+/\text{NO}_3^-$ and Si application on N ($P \leq 0.05$) and Si ($P \leq 0.05$) root accumulation (Table 3). Nitrogen and Si root accumulation decreased by 21.9% and 45.4%, respectively, in yellow passionfruit seedlings grown under high $\text{NH}_4^+/\text{NO}_3^-$ compared to plants under low $\text{NH}_4^+/\text{NO}_3^-$, regardless Si application (Table 3). In contrast, an increase on root N and Si accumulation by 13.4% and 20.0%, respectively, was observed in plants applied with Si,

Table 3. ANOVA of N, Si, K, Ca, and Mg root and shoot accumulation in yellow passionfruit seedlings under different NH₄⁺ and NO₃⁻ ratios with and without Si application.

Treatments	N		Si		K		Ca		Mg	
	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot
	mg plant ⁻¹									
NH ₄ ⁺ /NO ₃ ⁻ (R)	41.1**	23.5**	99.6**	0.2 ^{ns}	23.7**	9.1**	29.8**	23.2**	17.4**	14.7**
40/60	37.4	270.9	1.1	0.8	35.5	201.9	10.7	168.3	4.4	30.7
75/25	29.2	215.9	0.6	0.7	25.8	173.3	6.6	114.8	3.5	23.0
LSD	2.7	24.1	0.2	-	4.2	20.1	1.6	7.9	0.4	4.3
Silicon (Si)	10.2**	20.5**	6.9*	26.3**	2.1 ^{ns}	23.4**	1.8 ^{ns}	20.8**	2.9 ^{ns}	19.8**
Absence	31.2	217.7	0.8	0.5	29.2	164.6	8.1	116.3	3.8	22.4
Presence	35.3	269.1	1.0	0.9	32.1	210.5	9.1	166.8	4.1	31.3
LSD	2.7	24.1	0.1	0.2	-	20.1	-	7.9	-	4.3
R×Si	17.1*	0.1 ^{ns}	7.3*	1.6 ^{ns}	0.1 ^{ns}	0.1 ^{ns}	0.2 ^{ns}	1.5 ^{ns}	1.5 ^{ns}	0.8 ^{ns}
CV, %	8.6	10.4	10.4	13.8	14.6	11.3	19.3	17.5	11.8	16.8

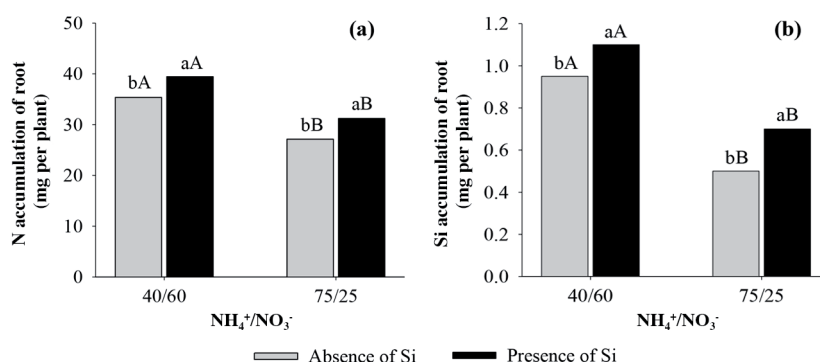
LSD: Least significant difference; CV: coefficient of variation; ^{ns}: nonsignificant.

*, **Significant at the 0.05 and 0.01 probability levels, respectively, according to F test.

regardless NH₄⁺/NO₃⁻ (Figures 3a and b). The reduction in N root accumulation in plants grown under high ammoniacal N might have resulted from the toxic effect of NH₄⁺ in plants, which causes damaging in the root system and reduction in nutrient uptake (Li et al., 2014). Silicon content in plants applied with Si was significantly higher than in plants without Si application, regardless NH₄⁺ concentration (Figure 3b). This demonstrates that yellow passionfruit is capable of taking up Si from nutrition solution and further accumulate it in its cells. The beneficial effect of Si is attributed to its deposition on plant root and shoots tissue as SiO₂, which enhances plant strength and act as a physical barrier against biotic and abiotic stresses (Ma and Yamaji, 2015). Plants that accumulates high concentration of Si in their cells are more prone to resist abiotic stresses, such as ammoniacal toxicity, than plant that do not accumulate Si in their cells (Ma and Yamaji, 2015).

Shoot N accumulation was significantly affected by NH₄⁺/NO₃⁻, as well as root and shoot K, Ca and Mg accumulation. Significant effect of Si application was also observed on N, Si, K, Ca and Mg shoot accumulation (Table 3). Root and shoot accumulation of K, Ca and Mg were lower in plants under high NH₄⁺ concentration than in plants grown under low NH₄⁺ concentration (Table 3). The reduction in accumulation of these cations in plants grown under high ammoniacal N was also observed by others author in *Fragaria* spp. (Choi et al., 2011), *Eustoma grandiflorum* (Mendoza-Villarreal et al., 2015), *Sorghum bicolor* (Chen et al., 2016), and *B. oleracea* (Barreto et al., 2017). The nutrient imbalance observed in the ammoniacal system is due to the antagonism effect between NH₄⁺ and the other cations, as they compete for the same

Figure 3. Nitrogen (a) and Si (b) root accumulation in yellow passionfruit seedlings under different NH₄⁺ and NO₃⁻ ratios with or without Si application.



Means followed by different lower-case letters indicate Si significant effect within NH₄⁺ and NO₃⁻ ratios and uppercase letters indicate NH₄⁺ and NO₃⁻ ratios significant effect within Si concentrations, according to Tukey's test (P ≤ 0.05).

uptake site in the roots (Marschner, 2012). In addition, there is an enhancement of NH_4^+ influx in the plasma membrane, which increase other cations efflux with further extrusion of these cations into the cell vacuole; thus, a deficiency symptoms expression in the plant (Mendoza-Villarreal et al., 2015).

Yellow passionfruit seedlings grown in the presence of the beneficial element showed higher K, Ca and Mg shoot accumulation than plants cultivated without Si application, regardless NH_4^+ concentration (Table 3). The beneficial effect of Si on cations uptake might vary according to plant species and stress conditions (Chen et al., 2016). Barreto et al. (2017) observed that Si increased K, Ca and Mg foliar content of brassicas cultivated in nutrient solution containing or not high ammoniacal N. Silicon stimulates H^+ -ATPase activity in cell membrane, which might explain its effect on K uptake (Liang, 1999). Moreover, Si reduces the concentration of reactive oxygen species in the plant, such as O_2 and H_2O_2 , which mitigates foliar chlorosis and necrosis symptoms resulted from K deficiency (Chen et al., 2016). The mechanisms by which Si increases Ca and Mg uptake is still being studied.

Plant growth, N use efficiency and dry matter

A significant interaction effect between $\text{NH}_4^+/\text{NO}_3^-$ and Si was observed on stem diameter, root length, NUE and root DM. Stem diameter and root length of plants cultivated under high $\text{NH}_4^+/\text{NO}_3^-$ without Si were lower than in plants cultivated under low $\text{NH}_4^+/\text{NO}_3^-$ (Table 4). Similar effect of NH_4^+ toxicity on plant growth was observed in *E. grandiflorum* (Mendoza-Villarreal et al., 2015), *C. sativus* (Campos et al., 2016) and *S. lycopersicum* (Barreto et al., 2016; 2018). In contrast, plants cultivated under high $\text{NH}_4^+/\text{NO}_3^-$ with Si application had 17% bigger stem diameter (Figure 4a) and 15.4% bigger root length (Figure 4b) than plants without Si application. Silicon application increased root N accumulation (Figure 3a) and leaf green color index (Figure 2a) of plants under ammoniacal stress, which might explain the better development of this plant parts. Moreover, plants were under lower stress as indicated by lower plant electrolyte leakage index (Figure 2b). The improvement on root system were also observed in *Z. mays*, grown in nutrient solution containing Si and high concentration of NaCl (Kochanová et al., 2014), and in *S. lycopersicum* (Barreto et al., 2016), and *B. oleracea* (Barreto et al., 2017) grown in nutrient solution with Si and high ammonium concentration. These results elucidate the beneficial effect of this element when plants are under abiotic stresses. It is noteworthy that in high concentrations of ammonium, Si promoted greater NUE, resulting in better use of nitrogen to increase stem diameter and root DM. In this case, no toxic effect of NH_4^+ was observed, even at high concentrations, probably due to the Si mitigating effect.

Individual effect of $\text{NH}_4^+/\text{NO}_3^-$ and Si was also observed on leaf area, NUE and shoot DM (Table 4). Regardless Si application, high $\text{NH}_4^+/\text{NO}_3^-$ reduced leaf area of yellow passionfruit seedlings by 27.1% compared to low $\text{NH}_4^+/\text{NO}_3^-$ (Table 4). The effect of high $\text{NH}_4^+/\text{NO}_3^-$ on leaf area is due to NH_4^+ toxicity in yellow passionfruit seedlings, which reduces N accumulation (Table 2); hence, limits leaf expansion.

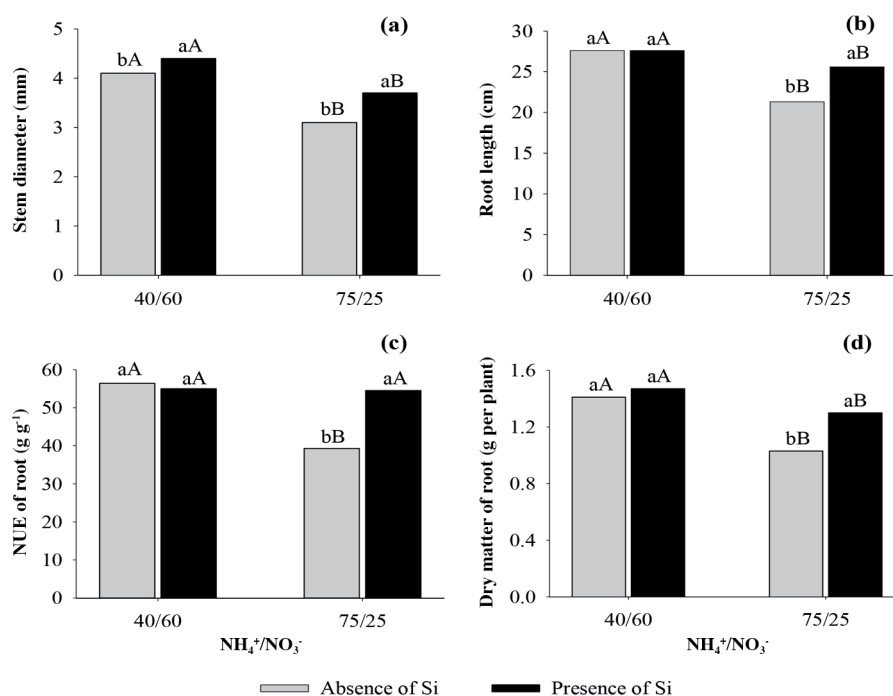
Table 4. ANOVA of plant height, stem diameter, leaf area, root length, and root and shoot nitrogen use efficiency (NUE) and dry matter of yellow passionfruit seedlings under different NH_4^+ and NO_3^- ratios with and without Si application.

Treatment	Stem diameter	Leaf area	Root length	NUE		Dry matter	
				Root	Shoot	Root	Shoot
	mm	cm ²	cm	— g g ⁻¹ —		— g plant ⁻¹ —	
$\text{NH}_4^+/\text{NO}_3^-$ (R)	192.6**	116.7*	58.8**	15.6**	40.4**	45.7**	31.5**
40/60	4.3	736.2	27.6	55.7	193.3	1.5	7.1
75/25	3.4	536.3	23.4	46.9	130.6	1.2	5.3
LSD	0.2	40.3	1.2	4.7	20.9	0.1	0.7
Silicon (Si)	53.2**	60.7**	15.8**	9.5**	13.9*	16.9**	12.3**
Absence	3.6	574.2	24.4	47.9	152.1	1.2	5.6
Presence	4.1	708.3	26.6	54.7	174.7	1.4	6.8
LSD	0.2	40.3	1.2	4.7	20.9	0.1	0.7
R×Si	7.2*	1.0 ^{ns}	15.8**	13.7**	0.1 ^{ns}	7.1*	0.2 ^{ns}
CV, %	3.7	6.5	4.7	9.7	13.6	6.9	11.7

LSD: Least significant difference; CV: coefficient of variation; ^{ns}: nonsignificant.

*, **Significant at the 0.05 and 0.01 probability levels, respectively, according to F test.

Figure 4. Stem diameter (a), root length (b), root nitrogen use efficiency (c) and root dry matter (d) of yellow passionfruit seedlings under different NH_4^+ and NO_3^- ratios with or without Si application.



Means followed by different lower-case letters indicate Si significant effect within NH_4^+ and NO_3^- ratios and uppercase letters indicate NH_4^+ and NO_3^- ratios significant effect within Si concentrations, according to Tukey's test ($P \leq 0.05$).

Root NUE and DM of plants cultivated under high concentration of NH_4^+ without Si application were decreased by 31.6% compared to plants with Si application (Figures 4c and 4d). High accumulation of NH_4^+ in the chloroplasts may block the metabolism of glutamine synthetase-glutamate synthase (GS-GOGAT) complex and reduce NUE, as this complex is responsible for N uptake in plants (Bittsánszky et al., 2015). Moreover, NH_4^+ toxicity decreased root NUE in plants under no application of Si due to its effect on nutrient uptake. These nutrients have physiological/structural enzymatic role in the plant that is involved on N conversion to DM, as elucidated by a positive correlation between root N efficiency and root accumulation of N ($r = 0.88^{**}$), K ($r = 0.78^{**}$), Ca ($r = 0.81^{**}$) and Mg ($r = 0.76^{**}$). Therefore, decreased root NUE is associated to decreased accumulation of these nutrients.

Regardless NH_4^+ and NO_3^- ratios, Si increased leaf area, root NUE and shoot DM (Table 4). An interaction between $\text{NH}_4^+/\text{NO}_3^-$ and Si demonstrated that under high $\text{NH}_4^+/\text{NO}_3^-$, Si enhanced stem diameter (Figure 4a), root length (Figure 4b), root NUE (Figure 4c) and root DM (Figure 4d) of yellow passionfruit seedlings. Enhanced shoot NUE due to Si application was also observed in *C. arietinum* (Kurdali et al., 2013), *Z. mays* (Campos et al., 2015), and *B. oleracea* (Barreto et al., 2017). It has been reported that Si supply to nutrient solution may positively affect nutrients uptake, such as N (Campos et al., 2016) and other cations (Barreto et al., 2017; Mantovani et al., 2018). Therefore, higher response in plant growth might be expected under this nutrient solution.

The beneficial effect of Si on shoot DM, regardless ammonium concentration in the nutrient solution (Table 4), is explained by its accumulation in plants. There is a positive correlation between Si shoot accumulation and shoot DM content ($r = 0.72^{**}$), as well as between Si root accumulation and DM content ($r = 0.61^{**}$). Therefore, increased shoot DM due to increased Si accumulation resulted in higher leaf area and stem diameter.

In addition, Si increased root DM production in seedlings of yellow passionfruit cultivated under high NH_4^+ (Figure 4d). This result might be due to Si higher accumulation in such conditions (Figure 3b) and higher root NUE (Figure 4c), elucidated by a positive correlation between root Si accumulation and root NUE ($r = 0.80^{**}$) and root Si accumulation and root DM ($r = 0.94^{**}$).

Therefore, Si application might be a management practice option to mitigate ammoniacal N toxicity in yellow passionfruit seedlings cultivation, especially in hydroponic system in which plants are prone to nutritional stresses. Further studies regarding Si role in the mitigation of ammonium toxicity in other fruit plants is necessary, since the genetic factor might be taken into consideration.

CONCLUSIONS

Silicon application in yellow passionfruit seedlings under high level of ammonium increased N accumulation and N use efficiency, root length and root DM, and leaf green color index. In addition, Si application reduced leaf electrolyte leakage index, which resulted in higher stem diameter. These results prove that silicon application mitigates ammonium toxicity in yellow passionfruit seedlings.

ACKNOWLEDGEMENT

We are grateful to the National Council for Scientific and Technological Development (CNPq) for the doctoral scholarship granted to the first author.

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