



AMMONIA TOXICITY AFFECTS CATIONS UPTAKE AND GROWTH IN PAPAYA PLANTS INCLUSIVE WITH SILICON ADDITION

Toxicidad amoniaca afecta la absorción de cationes y el crecimiento de plantas de papaya inclusive con adición de silicio

Bruna SANTOS SILVA¹, Renato de MELLO PRADO², Alexander CALERO HURTADO²*, Renata APARECIDA DE ANDRADE³, Gilmara PEREIRA DA SILVA⁴.

¹Departamento de Fitotecnia e Zootecnia. Universidade Estadual do Sudoeste da Bahia (UESB). Estrada do Bem Querer, Km 04, Caixa Postal 95, CP: 45083-900. Vitória da Conquista, Bahia, Brasil

²Departamento de Ciências da Produção Agrícola, Setor de Solos e Adubos. Universidade Estadual Paulista (UNESP). Via de acesso Prof. Paulo Donato Castellane s/n, CP: 14884900, Jaboticabal, São Paulo, Brasil

³Departamento de Ciências da Produção Agrícola, Setor de Produção Vegetal. Universidade Estadual Paulista (UNESP). Avenida Prof. Paulo Donato Castellane s/n, CP: 14884900, Jaboticabal, São Paulo, Brasil.

⁴Departamento de Solos. Universidade do Estado de Mato Grosso (UNEMAT). Via de acesso Perimetral Rogério Silva - Norte 2, CP: 78580-000. Alta Floresta, Mato Grosso, Brasil.

*For correspondence: alexcalero34@gmail.com

Received: 04th May 2019, Returned for revision: 31st August. 2019, Accepted: 08th November 2019.

Associate Editor: Hernán Romero.

Citation/Citar este artículo como: Silva BS, Prado RM, Calero Hurtado A, Andrade RA, Silva GP. Ammonia toxicity affects cations uptake and growth in papaya plants inclusive with silicon addition. Acta Biol Colomb. 2020;25(3):345-353. Doi: <http://dx.doi.org/10.15446/abc.v25n3.79490>

ABSTRACT

High ammonia (NH_4^+) concentration can exert stress on many plants, which causes nutritional disorders and reduction on plant growth. However, depending on the intensity of the stress, it may be attenuated by silicon. In this work, the response of impact of cations and silicon accumulations and plant growth in cultivated papaya plants was investigated under different toxic ammonia concentrations regardless of the presence of silicon (Si). The experiment was conducted at the Universidade Estadual Paulista (UNESP) with papaya seedlings, variety 'Grupo Formosa' (Calimosa híbrida 01), grown in a glass greenhouse, in 1.7 dm³ pots filled with pine and coconut fiber-based substrate. The experimental design was a randomized block design, in a 5 × 2 factorial arrangement. There were five ammonia concentrations: 10, 20, 40, 80, and 100 mmol L⁻¹ that were delivered via nutrient solution, in the absence and presence of Si (2 mmol L⁻¹), with five replicates. After 31 days of growth, the cations and silicon accumulations in the shoot, plant height, stem diameter, root, and shoot dry matter were evaluated. Results revealed that increased ammonia concentration showed toxicity in papaya plants and stronger reductions in Ca, Mg, K and Si accumulations, plant heights, stem diameters, and root and shoot dry matter production, even when silicon was present and with greater effects on the shoot dry matter (87 %) than that of the roots (13 %).

Keywords: *Carica papaya*, biomass, phytotoxicity, nitrogen, nutrients .

RESUMEN

Las altas concentraciones de amonio (NH_4^+) pueden ejercer estrés en las plantas cultivadas, lo que causa trastornos nutricionales y reducción del crecimiento. Sin embargo, dependiendo de la intensidad del estrés, este puede atenuarse mediante el silicio (Si). En este trabajo, se investigó la respuesta de la acumulación de cationes y silicio y el crecimiento de plantas de papaya cultivadas en diferentes concentraciones tóxicas de amonio independientemente de la presencia de silicio. El experimento se realizó en la Universidade Estadual Paulista (UNESP), con plántulas de papaya, variedad Grupo Formosa (Calimosa híbrida 01), cultivadas en invernadero, en macetas de 1,7 dm³, rellenas con sustrato a base de fibra de pino y coco. El diseño experimental fue en bloques al azar, en esquema factorial 5×2, con cinco concentraciones de amonio 10, 20, 40, 80 y 100 mmol L⁻¹, en la ausencia y presencia de Si (2 mmol L⁻¹), con cinco repeticiones. A los 31 días posteriores del inicio de los tratamientos, se evaluó la acumulación de calcio, magnesio, nitrógeno, potasio y silicio, altura de la planta, diámetro del tallo y la materia seca de la raíz y los brotes. Los resultados revelaron que el aumento de la concentración de amonio mostró toxicidad en plantas de papaya y una reducción en la acumulación de calcio, magnesio, potasio y silicio, la altura de la planta, diámetro del tallo y la producción de materia seca de raíces y brotes, aunque el silicio esté presente, con mayor afectación en la materia seca de los brotes (87 %) que en las raíces (13 %).

Palabras clave: *Carica papaya* L., biomasa, fitotoxicidad, nitrógeno, nutrientes.



INTRODUCTION

Nitrogen (N) is absorbed in nitrate (NO_3^-) or ammonia (NH_4^+) form, is the second most-required element by papaya plants [*Carica papaya* (Linn)], and it is important to meet its demand throughout the plant life cycle (Neto *et al.*, 2011). Once absorbed, NH_4^+ can be quickly used in the synthesis of amino acids and other organic nitrogen compounds (Bybordi, 2010), and there is no need to be reduced to nitrate, avoiding energy waste. However, excess NH_4^+ can affect the development of the plants (Bittsánszky *et al.*, 2015; Esteban *et al.*, 2016). Most species of plants develop toxicity symptoms when subjected to high concentrations of NH_4^+ . This may lead to decreased photosynthesis, lower growth and yield, lower cation content, and variation in the content of metabolites such as amino acids or organic acids and rhizosphere acidification (Borgognone *et al.*, 2013; Esteban *et al.*, 2016), although this ion is an intermediate in several metabolic reactions (Li *et al.*, 2014).

Different physiological and biochemical hypotheses may explain the action of NH_4^+ in plants. These include acidification of the growth medium and NH_4^+ -toxicity per se leading to antagonism of cation uptake, and alterations in the osmotic balance (Britto and Kronzucker, 2002; Horchani *et al.*, 2010; Nasraoui-Hajaji and Gouia, 2014). The pH of the rooting medium is of paramount importance for plant growth, as a large number of processes (e.g., nutrient availability, uptake rate and availability of toxic ion species) are closely related to this parameter (Marschner, 2012). A consequence of acidification is the lower availability of cations, such as K, Ca and Mg (Ahmad *et al.*, 2006).

The damaging effects of NH_4^+ nitrogen contrast themselves with the beneficial effects of Si (Campos *et al.*, 2015), because this beneficial element increases the growth of many plants under abiotic stresses from different mechanisms (Bybordi, 2010). In addition, in unstressed plants it affects the increase of nitrogen metabolism, enabling increases in fresh mass, seed production, photosynthesis rates, chlorophyll contents, and the activity of nitrate reductase (Bybordi, 2012).

Studies involving the use of Si as a possible attenuator of the deleterious effects caused by excess NH_4^+ have been carried out for some crops. These have included benefits to rice (Ávila *et al.*, 2010), corn (Campos *et al.*, 2015), cucumber (Campos *et al.*, 2016) and tomato (Barreto *et al.*, 2016), cauliflower and broccoli (Barreto *et al.*, 2017), radish (Olivera *et al.*, 2017), and beets (Viciedo *et al.*, 2019); however, there was no effect for other crops, such as plum trees (Ferreira *et al.*, 2013). These effects of NH_4^+ toxicity vary from species to species with probable distinctions in the damage of the growth of the aerial parts in relation to the roots, due to the differentiated cation accumulations of these organs, and it may depend on the Si accumulation in the species studied. It is hoped that Si is more important

in attenuating NH_4^+ toxicity for species that have greater uptake of Si.

Given the need for a better understanding of Si and NH_4^+ interactions, our hypothesis is that NH_4^+ toxicity could be attenuated with the use of Si, depending on its intensity. Additionally, the NH_4^+ toxicity in papaya plants induces more damage in shoots than roots, given the possible greatest restriction of cation uptake and reduction of plant growth. Therefore, the purpose of this research was to investigate the response of cations, Si accumulations, and plant growth impacts of different NH_4^+ concentration in papaya plants when Si is present.

MATERIAL AND METHODS

The experiment was carried out in a greenhouse at Sao Paulo State University (UNESP) – Campus of Jaboticabal, Brazil, of January to March 2015. The experimental design was in randomized blocks in a 5×2 factorial arrangement, with five NH_4^+ concentrations (10, 20, 40, 80, and 100 mmol L^{-1}), in the absence and presence of Si (2 mmol L^{-1}), with five replications. Each experimental unit consisted of a seedling cultivated in a polypropylene pot (1.7 dm^3).

The experiment was conducted on papaya seedlings (*Carica papaya* L., ‘Grupo Formosa’ (Calimosa híbrida 01)). The nutrient solution proposed by Hoagland and Arnon (1950) was used, by modifying the source of iron for Fe-EDDHMA. The nitrogen source used to compose the treatments was ammonia chloride (NH_4Cl) solution, and the Si source was monosilicic acid. The pH of the Hoagland nutrient solution was monitored daily and maintained at 5.5 ± 0.5 using 1 N hydrochloric acid (HCl) solutions.

Sowing of papaya was carried out in polyethylene trays, with a capacity of 1.5 dm^3 , which was filled with a commercial substrate based on pine and coconut fiber, and a seed was sown in each container. Irrigation was daily, and substrate moisture was kept near the maximum water retention. After emergence (22 days), the papaya seedlings were transplanted to polypropylene pots, keeping the same substrate mentioned above. Thereafter, over seven days, applications of the Si solution (2 mmol L^{-1}) were performed, using 20 mL per container only for the treatments with Si and a complete nutritive solution with N as nitrate in all treatments. After seven days, the treatment with nutritive solutions containing NH_4^+ began. For each pot, a volume of 50 mL of the solutions was applied in the first 15 days; with the growth of the plants, that volume increased to 100 mL, which was applied daily according to the respective treatments.

The vessels used contained at the bottom a collecting container to return the nutritive solution, thus avoiding its loss. The collection of the nutritive solution and new application to the pots were performed in the early morning between 8:00 to 9:00 am. After applying the treatments, the

plants were observed daily, and at the time of the onset of the visual symptoms of NH₄⁺ toxicity, the descriptions of the symptoms, the images of the plants, and the dates of each observation were recorded.

After 31 days of treatment applications, the height of the seedlings (cm) were determined, from the base of the seedlings to the insertion of the first fully developed leaf, with support of graduated rule; and the stem diameter (mm), 5 cm above the base of the seedlings, was measured with a digital caliper. After plants were collected, they were washed with distilled water to remove residues. Then the plants were washed with a detergent solution (0.2 %), hydrochloric acid solution (0.1 %), and finally deionized water (Prado, 2020). The seedlings were separated into roots and shoots, and then they were placed in paper sacks and dried in a forced-air oven (Tecnal® TE 394-3-Brazil) at a constant temperature of 65° C until they reached a constant weight, and then the root dry matter (RDM) and shoot dry matter (SDM) (mg per plant) were obtained.

After obtaining the dry matter weights, the samples of the shoots were then pulverized with a Wiley mill fitted with a stainless-steel chamber and blades (Marconi® MA 360, SCompany, China). The concentrations of N, K, Ca and Mg were determined according to the methodology proposed by Bataglia *et al.* (1983), and Si was determined according to Kraska and Breitenbeck (2010). Total N, K, Ca, Mg and Si accumulation in shoots were calculated as the N, K, Ca, Mg, and Si concentrations per total plant dry weight (mg per plant) (Lawlor, 2002).

The data obtained were submitted to variance analysis (ANOVA), using the F test at 5 % of probability. When significant for the averages of the source of variation of NH₄⁺ doses, the polynomial regression analysis was carried out. The appropriate regression equation was selected on the basis of higher R² values. The study of the relationships between the variables was performed by means of Pearson correlation (r). For the statistical analysis, the software Assistat 7.7 beta was used.

RESULTS

We found significant ($p \leq 0.05$) main differences in NH₄⁺ concentration and no interaction effects of NH₄⁺ and Si application on Ca, Mg, K, N, and Si accumulation in papaya plants (Figs. 1a-e). The increase of NH₄⁺ concentration in the nutritive solution origin decreased linearly in the accumulations of Ca, Mg, K, and Si in the shoots of papaya seedlings (Figs. 1a, b, c, and e), regardless of the presence of Si, but it did not affect the shoot N accumulation.

There was a significant ($p \leq 0.05$) effect of NH₄⁺ concentration and no interaction effect of NH₄⁺ and Si on plant growth (Figs. 2a-d). In papaya plants, the plant heights (Fig. 2a) and stem diameters (Fig. 2b) decreased

linearly with the increase of NH₄⁺ concentration supplied via nutritive solution, regardless of the presence of Si.

There were significant differences ($p \leq 0.05$) for NH₄⁺ concentrations and no interaction between Si and NH₄⁺ levels on root and shoot dry matter production in papaya plants (Figs. 2c and d). In papaya plants, the increase of NH₄⁺ concentration showed a linear decreased in RMD (Fig. 2c) and in SDM (Fig. 1d), even when Si was present.

A significantly high correlation ($p \leq 0.01$) was observed among all variables evaluated (Table 1). The NH₄⁺ toxicity induced decreases in dry matter production in shoots, especially with the strong correlation with the reduction of chemical element accumulations, such as K ($r = 0.95^{**}$), total N ($r = 0.94^{**}$) and Mg and Si ($r = 0.93^{**}$) and by lower accumulations of RDM ($r = 0.90^{**}$) (Table 1).

DISCUSSION

EFFECT OF NH₄⁺ ON CATIONS AND SILICON ACCUMULATION

The overall results of this experiment suggest that 20 mmol·L⁻¹ of NH₄⁺ supplementation is enough to cause toxicity in papaya plants. Many plant species have been characterized as NH₄⁺ tolerant or sensitive (Cruz *et al.*, 2011). This study demonstrated that papaya plants are very sensitive to NH₄⁺ application and showed a decrease of cations and Si accumulations (Figs. 1a-e), which led to a consequent reduction in the plant growth, even when Si was present. Therefore, the use of Si in nutrient solution did not affect papaya plant growth with or without ammonia toxicity (Figs. 2a-d).

In papaya seedlings, increasing of NH₄⁺ concentrations significantly decreased the Ca, Mg, K and Si accumulations (Figs. 1a, b, d, and e), which caused significant reductions in plant growth (Figs. 2a-d), and high correlations among these variables (Table 1). In contrast, the exclusive supply of N as NH₄⁺ is harmful to many plants and may cause ion imbalances (Szczerba *et al.*, 2008; Domínguez-Valdivia *et al.*, 2008). Recent studies have indicated that high concentrations of NH₄⁺ decrease the accumulation of Ca and Mg in tomato plants (Borgognone *et al.*, 2013). This is corroborated by a acidification of the rhizosphere leading a lower cation content, changes of several metabolite levels, leaf chlorosis, net photosynthesis decreases (such as amino acids or organic acids), and lower plant yields (Britto and Kronzucker, 2002).

An excess in NH₄⁺ is reflected in the integrated phenotypic symptoms, such as reduced plant growth, changes in root architecture, decreases in the root/shoot ratios, and leaf chlorosis, among others (Bittsánszky *et al.*, 2015; Esteban *et al.*, 2016). This causes the inhibition of cations K, Mg or Ca uptake and consequent changes in plant ion balance, intra-cellular alkalization and extracellular acidification,

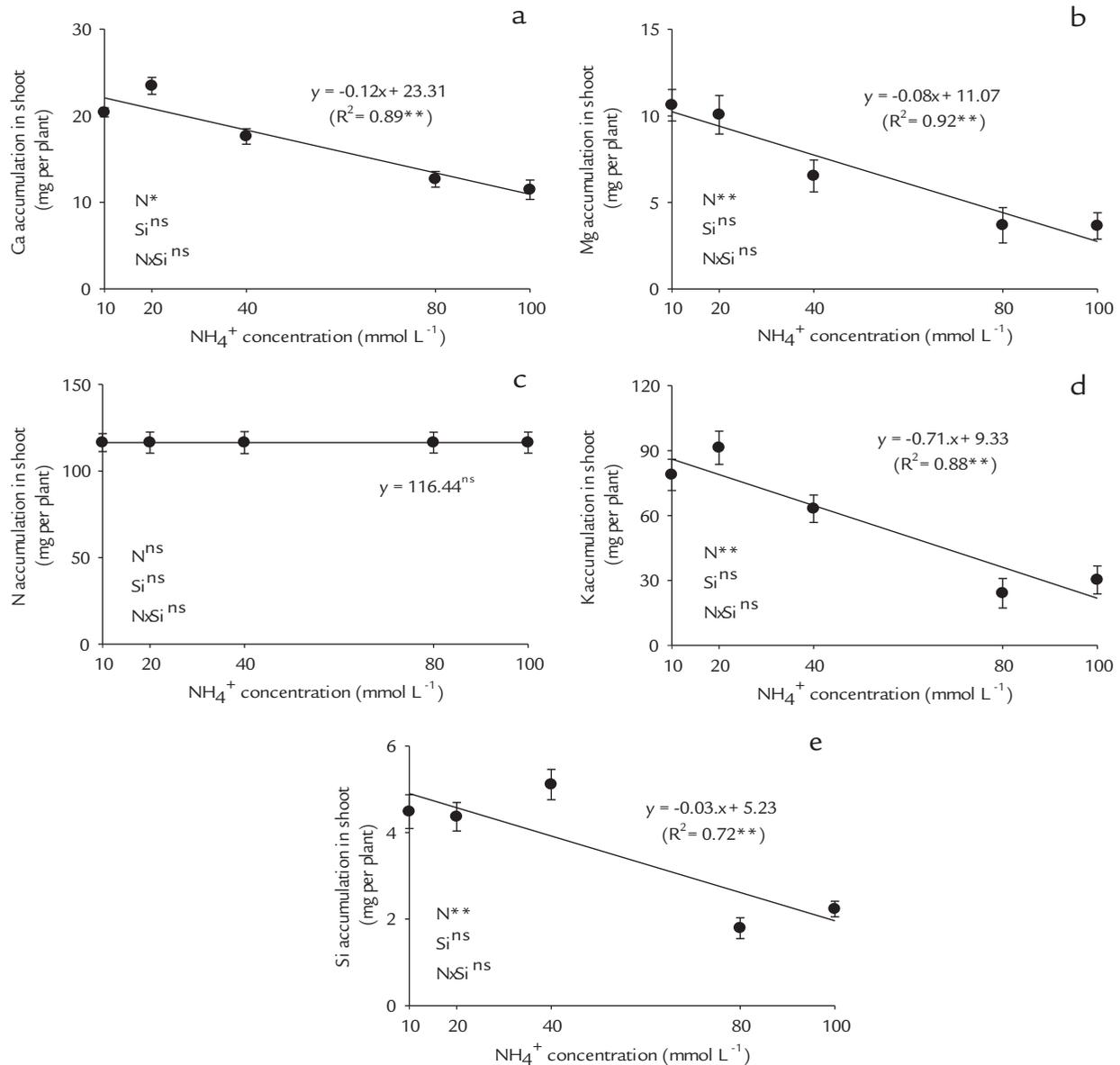


Figure 1 Cation accumulations in the shoots of papaya plants. Calcium accumulation (a), magnesium accumulation (b), nitrogen accumulation (c), potassium accumulation (d) and silicon accumulation (e), as a function of five NH_4^+ concentrations, 10, 20, 40, 80 and 100 mmol L^{-1} , in nutritive solutions in the absence and presence of Si (2 mmol L^{-1}). *Significant at $p \leq 0.05$ of probability of error **Significant at $p \leq 0.01$ of probability of error; ns-insignificant by the F test at $p \leq 0.05$.

the inhibition of root respiration and stimulation of photorespiration, interference with photosynthetic activity, and altered expression/activity of NH_4^+ (Cruz *et al.*, 2011; Ariz *et al.*, 2011; Esteban *et al.*, 2016).

On the other hand, the decreased cation and Si uptake and accumulation in shoots of papaya plants can be attributed to the drastic reduction in root dry weight, which has also been observed in other sensitive species, such as *Arabidopsis* sp. (Li *et al.*, 2014). The severity of NH_4^+ toxicity is increased when K is in a low concentration in the production system. It is important for NH_4^+ assimilation and in the formation of the amino acids (Ten Hoopen *et al.*, 2010;

Li *et al.*, 2014). This situation did not occur in the present study, since an adequate concentration of K was used in the nutritive solution. Recent studies have indicated that NH_4^+ excess caused toxicity and ion imbalance in different species, such as wheat (Britto *et al.*, 2001; Szczerba *et al.*, 2008), plum trees (Ferreira *et al.*, 2013), tomato (Von Wiren *et al.*, 2000; Barreto *et al.*, 2016), cauliflower and broccoli (Barreto *et al.*, 2017), radish (Olivera *et al.*, 2017), and more recently in sugar beet (Viciedo *et al.*, 2019).

The presence of Si in the nutritive solution did not affect the accumulation of K in the shoots of papaya plants (Fig. 1d). Different results were obtained in canola by Bybordi (2010),

Table 1. Correlations between the evaluated variables in papaya plants cultivated in nutritive solutions.

VA/VA	Ca ¹	Mg ²	N ³	K ⁴	Si ⁵	PH ⁶	SD ⁷	SDM ⁸	RDM ⁹
Ca	1	0.90	0.85	0.87	0.74	0.80	0.85	0.89	0.84
Mg	**	1	0.81	0.95	0.83	0.80	0.85	0.93	0.89
N	**	**	1	0.85	0.86	0.82	0.80	0.94	0.84
K	**	**	**	1	0.86	0.85	0.86	0.95	0.86
Si	**	**	**	**	1	0.85	0.79	0.94	0.82
PH	**	**	**	**	**	1	0.95	0.89	0.84
SD	**	**	**	**	**	**	1	0.88	0.91
SDM	**	**	**	**	**	**	**	1	0.90
RDM	**	**	**	**	**	**	**	**	1

¹ accumulation of Ca; ² accumulation of Mg; ³ accumulation of N; ⁴ accumulation of K; ⁵ accumulation of Si; ⁶ plant heights; ⁷ stem diameters; ⁸ shoot dry matter; ⁹ root dry matter. Pearson correlation coefficients and their significance are given as ** $p \leq 0.01$.

who observed an increase in K accumulation in the presence of Si without the occurrence of stress. The difference among results was due to the NH₄⁺ toxicity in the present work inhibiting the interaction Si and K, and this situation did not occur in the cited work.

It has been reported that NH₄⁺ toxicity can decrease Ca and Mg uptake. Such findings have also been demonstrated in other recent studies of different plant species, such as *Hydrilla verticillata* (Wang *et al.*, 2010), *Egeria densa* (Su *et al.*, 2012), and tomato (Borgognone *et al.*, 2013).

In the present study, the Si accumulation was decreased according to increasing NH₄⁺ concentrations, even when Si was present in the nutritive solution (Fig. 1e). This presupposes that the Si supply did not promote the cation uptake, and had been reported previously in rice plants (Mauad *et al.*, 2003). One possible explanation for the reported findings is that papaya plants are dicots and are considered a non-Si-accumulating plant (Hodson *et al.*, 2005). However, some dicots, such as cucumber, melon, strawberry and soybean take up Si passively (Mitani and Ma, 2005), and tomato and beans exclude Si from uptake (Liang *et al.*, 2005; 2007). Recently, in dicotyledonous plants, the uptake of monosilicic acid (MSA) has been determined to be mediated by a process of diffusion, resulting in significantly lower MSA concentrations in the xylem compared to monocots (Mitani and Ma, 2005).

EFFECT OF NH₄⁺ ON PLANT GROWN

To avoid toxicity, plants need to maintain a fine balance between the uptake, production, and consumption of NH₄⁺ (Bittsánszky *et al.*, 2015). This leads to the question of the nature of the first response of plants to high levels of NH₄⁺ (Esteban *et al.*, 2016). Therefore, the use of Si in the nutritive

solution did not affect the growth of the papaya plants with or without NH₄⁺ toxicity. A similar result was observed by Ferreira *et al.* (2013) for plum trees, in which Si did not promote the growth of the plants. The low importance of Si for papaya plants was possibly because the plant is not accumulative of the element, a classification that covers most dicotyledons (Hodson *et al.*, 2005).

In this study, the excess NH₄⁺ affected the root dry weight, even when Si was present (Fig. 2c), this could be affected by the cation and Si uptake (Figs. 1a, b, d, and e). Our results corroborate the work done by Borgognone *et al.* (2013), in which the root dry matter of tomato decreased with the increase of NH₄⁺ dose, from the concentration of 0.021 mmol L⁻¹. This is possible because, in NH₄⁺ sensitive plants, such as papaya, the plant growth was affected. A similar effect had been previously reported in *Arabidopsis* sp., in which shoots tended to be the parts of the plant most sensitive to NH₄⁺ (Li *et al.*, 2014). In contrast, the roots constitute the first NH₄⁺ sensor, and the initial signals of NH₄⁺ toxicity appear at the root level, with a severe modification of the root system architecture. Commonly observed modifications include shorter primary root systems, the inhibition of root elongation, and embracing primary and lateral roots (Rogato *et al.*, 2010; Esteban *et al.*, 2016).

Depending on the dosage, the exclusive supply of N as NH₄⁺ may cause poor biomass accumulation (Szczerba *et al.*, 2008; Domínguez-Valdivia *et al.*, 2008). It has been observed that excess NH₄⁺ in nutritive solutions resulted in the growth suppression of corn plants (Campos *et al.*, 2015) and tomato plants (Borgognone *et al.*, 2013), which occurred with NH₄⁺ concentrations in the nutritive solution from 30 mmol L⁻¹ and 1 mmol L⁻¹, respectively. These results differ from those presented by (Mendoza-Villarreal *et al.*, 2015), in which the application of 15 mmol L⁻¹ of N with different NH₄⁺ proportions promoted an increase in the stem diameters and heights of lisianthus plants (*Eustoma grandiflorum* (Raf.) Shinn), according to the increase of NH₄⁺ proportion in the nutritive solution.

On the other hand, in sunflower plants, applying the concentration of 210 mg L⁻¹ of N in the NH₄⁺ form, resulted in a decrease in the dry matter production of the plants (Silva *et al.*, 2010). NH₄⁺ caused a reduction in the development of *Salvinia cucullata* under concentrations of 10 and 15 mmol L⁻¹, making the roots smaller and atrophied (Jampeetong *et al.*, 2012). Probably the growth inhibition has been related previously by Britto *et al.* (2001). Britto and Kronzucker (2002) and Von Wiren *et al.* (2000) verified that the futile transmembrane cycle of NH₄⁺ uptake and efflux through cell roots may carry a high energetic cost and be responsible for detrimental effects.

It was shown by the angular coefficients of the regression line that each 1 mmol L⁻¹ of NH₄⁺ added to the solution reduced the SDM by 0.0285 g per plant and the RDM by 0.0042 g per plant, totaling 0.0327 g per plant. The highest

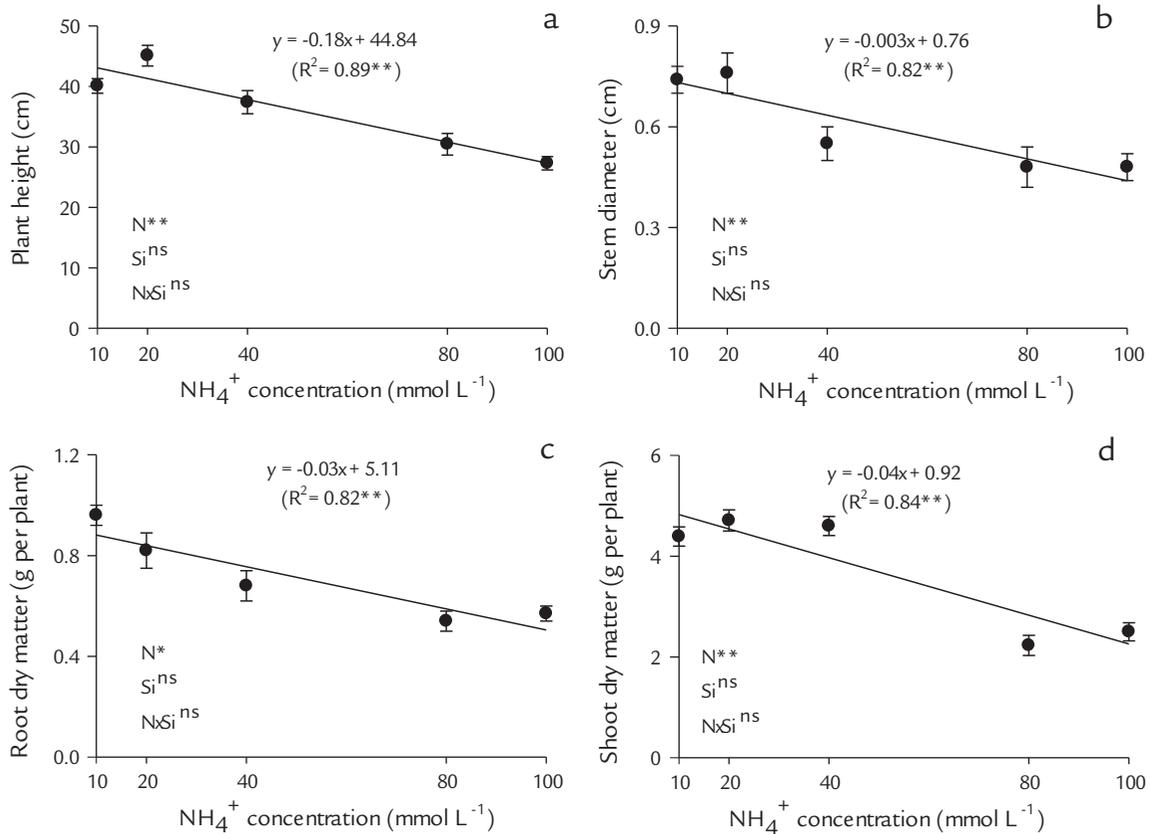


Figure 2 Plant growth characteristics in papaya seedlings, plant height (a), stem diameter (b), root dry matter (c) and shoot dry matter (d), as a function of five NH_4^+ concentrations, 10, 20, 40, 80 and 100 mmol L^{-1} , in nutritive solutions in the absence and presence of Si (2 mmol L^{-1}). ** Significant at $p \leq 0.01$ of probability of error; ns-insignificant by the F test at $p \leq 0.05$. Table 1. Correlations between the evaluated variables in papaya plants cultivated in nutritive solutions.

proportion of this decrease in the plant dry matter induced by NH_4^+ toxicity occurred for SDM (87 %) as compared with the RDM (13 %) (Figs. 2c and d). This greater loss of dry matter accumulation of the aerial part in relation to the plant roots under NH_4^+ stress is due to a slight restriction of root of cation uptake that easily reaches the aerial parts without having accumulated in the plant roots. Excess NH_4^+ in the aerial parts increases the contents of oxygen (O_2) and hydrogen peroxide (H_2O_2), inducing oxidative stress, lower contents of carotenoids (Wang *et al.*, 2010), lower photosynthetic rates, stomatal conductance (Borgognone *et al.*, 2013), and increases in ethylene synthesis (Britto and Kronzucker, 2002), reflected in the decrease in the dry matter of aerial parts.

Symptoms of NH_4^+ toxicity in papaya plants were characterized by the necrosis of older leaves, yellowing, and death of leaves, similar to that described by Esteban *et al.* (2016). These symptoms were evident from the 40 mmol L^{-1} NH_4^+ concentration. Similar results for toxicity were observed by Holzschuh *et al.* (2011) in rice plants at a 5 mmol L^{-1} NH_4^+ concentration in the nutritive solution. Differences in tolerances of the species to the toxicity depend not only

on genetic factors, but also on the concentrations of other nutrients in the nutritive solution.

It is also worth mentioning that increasing ammonium concentrations in nutrient solutions resulted in increased Cl ions, reaching 100 mmol L^{-1} , and this could have aggravated the ammonia toxicity. However, plants have high chlorine tolerance, may have in their tissues 20 to 200 times more than adequate levels without causing toxicity (Malavolta *et al.*, 1997), but there may be differences between species. According to Greenway and Munns (1980), dicotyledonous species continue their growth at concentrations between 250–500 mmol L^{-1} of Cl, presenting an optimal growth near 250 mmol L^{-1} . While for papaya plants, information is restricted for the Cl concentrations in nutrient solutions that impair its growth.

Thus, our results confirm the hypothesis that increased concentration of NH_4^+ in the nutrient solution resulted in a deleterious effect in ion balance and papaya seedlings even when silicon was present. This study provides the evidence that effect of Si supplied in nutrient solution alone did not attenuate the NH_4^+ toxicity in this sensitive species. There is, however, the possibility of studying the combined root and

foliar applications of Si, to increase the Si accumulation in non-accumulating plants and possible benefits.

CONCLUSIONS

The results confirmed that ammonia applied in high concentration markedly adversely affected the uptake of cations and plant growth of papaya plants. Consequences revealed from increased ammonia concentrations showed toxicity in papaya plants and stronger reduction in Ca, Mg, K and Si accumulations, plant heights, stem diameters, and root and shoot dry matter productions even when silicon was present. Although all levels of added ammonia effectively increased the deleterious effects on papaya plants, there was a greater effect on the shoot dry matter (87 %) than on the root dry matter (13 %).

ACKNOWLEDGMENT

We thank the Coordenação de Aperfeiçoamento do Pessoal de Nível Superior (CAPES/PROCAD), Universidade Estadual do Sudoeste de Bahia (UESB) and to the Universidade Estadual Paulista “Júlio de Mesquita Filho”, Campus of Jaboticabal for their technical support in the project.

DISCLOSURE OF INTEREST

The authors declare that there is no conflict of interest.

REFERENCES

- Ahmad A, Mohd S, Ismail MR, Yusop MK, Mahmood M. Effects of nitrogen forms on the growth and ionic content of lowland cauliflower under tropical greenhouse. *Acta Hortic.* 2006; 710:383–390. Doi: <https://doi.org/10.17660/ActaHortic.2006.710.46>
- Ariz I, Artola E, Asensio AC, Cruchaga S, Aparicio-Tejo PM, Moran JF. High irradiance increases NH₄⁺ tolerance in *Pisum sativum*: Higher carbon and energy availability improve ion balance but not N assimilation. *J Plant Physiol.* 2011;168(10):1009–1015. Doi: <https://doi.org/10.1016/j.jplph.2010.11.022>
- Ávila FM, Baliza DP, Faquin V, Araújo JL, Ramos SJ. Interação entre silício e nitrogênio em arroz cultivado sob solução nutritiva. *Rev Cienc Agron.* 2010;10(2):184–190. Doi: <https://doi.org/10.1590/S1806-66902010000200003>
- Barreto RF, Júnior AAS, Maggio MA, Prado RM. Silicon alleviates ammonium toxicity in cauliflower and in broccoli. *Sci Hortic.* 2017;225:743–750. Doi: <https://doi.org/10.1016/J.SCIENTA.2017.08.014>
- Barreto RF, Prado RM, Leal AJF, Troleis MJB, Junior GBS, Monteiro CC, *et al.* Mitigation of ammonium toxicity by silicon in tomato depends on the ammonium concentration. *Acta Agric Scand.* 2016;66(6):483–488. Doi: <https://doi.org/10.1080/09064710.2016.1178324>
- Bataglia OC, Teixeira JPF, Furlani PR, Furlani AMC, Gallo JR. Métodos de análise química de plantas. 1st ed. Campinas: Instituto Agronômico de Campinas; 1983. 31 p.
- Bittsánszky A, Pilinszky K, Gyulai G, Komives T. Overcoming ammonium toxicity. *Plant Sci.* 2015;231:184–190. Doi: <https://doi.org/10.1016/J.PLANTSCI.2014.12.005>
- Borgognone D, Colla G, Roupheal Y, Cardarelli M, Rea E, Schwarz D. Effect of nitrogen form and nutrient solution pH on growth and mineral composition of self-grafted and grafted tomatoes. *Sci Hortic.* 2013;149:61–69. Doi: <https://doi.org/10.1016/J.SCIENTA.2012.02.012>
- Britto DT, Kronzucker HJ. NH₄⁺ toxicity in higher plants: a critical review. *J Plant Physiol.* 2002;159(6):567–584. Doi: <https://doi.org/10.1078/0176-1617-0774>
- Britto DT, Siddiqi MY, Glass ADM, Kronzucker HJ. Futile transmembrane NH₄⁽⁺⁾ cycling: a cellular hypothesis to explain ammonium toxicity in plants. *Proc Natl Acad Sci USA.* 2001;98(7):4255–4258. Doi: <https://doi.org/10.1073/pnas.061034698>
- Bybordi A. Effect of ascorbic acid and silicon on photosynthesis, antioxidant enzyme activity, and fatty acid contents in canola exposure to salt stress. *J Integr Agric.* 2012;11(10):1610–1620. Doi: [https://doi.org/10.1016/S2095-3119\(12\)60164-6](https://doi.org/10.1016/S2095-3119(12)60164-6)
- Bybordi A. Influence of NO₃:NH₄ ratios and silicon on growth, nitrate reductase activity and fatty acid composition of canola under saline conditions. *African J Agric Res.* 2010;5(15):1984–1992.
- Campos CNS, Prado RDM, Roque CC, Neto AJL, Marques LJP, Chavez AP, *et al.* Use of Silicon in mitigating ammonium toxicity in maize plants. *Am J Plant Sci.* 2015;6:1780–1784. Doi: <https://doi.org/10.4236/ajps.2015.611178>
- Campos CNS, Prado RDM, Caione G, Neto AJDL, Mingotte LFLC. Silicon and excess ammonium and nitrate in cucumber plants. *African J Agric Res.* 2016;11(4):276–283. Doi: <https://doi.org/10.5897/AJAR2015.10221>
- Cruz C, Domínguez-Valdivia MD, Aparicio-Tejo PM, Lamsfus C, Bio A, Martins-Loução Ma, *et al.* Intra-specific variation in pea responses to ammonium nutrition leads to different degrees of tolerance. *Environ Exp Bot.* 2011;70(2-3):233–243. Doi: <https://doi.org/10.1016/J.ENVEXPBOT.2010.09.014>
- Domínguez-Valdivia MD, Aparicio-Tejo PM, Lamsfus C, Cruz C, Martins-Loução Ma, Moran JF. Nitrogen nutrition and antioxidant metabolism in ammonium-tolerant and -sensitive plants. *Physiol Plant.* 2008;132(3):359–369. Doi: <https://doi.org/10.1111/j.1399-3054.2007.01022.x>
- Esteban R, Ariz I, Cruz C, Moran JF. Review: Mechanisms of ammonium toxicity and the quest for tolerance. *Plant Sci.* 2016;248:92–101. Doi: <https://doi.org/10.1016/J.PLANTSCI.2016.04.008>
- Ferreira SGM, Botelho RV, Duarte C, Ferrari M, Zaluski WL. Desenvolvimento e fitossanidade de ameixeiras tratadas com silício em sistema orgânico. *Rev Bras Frutic.* 2013;35(4):1059–1065. Doi: <https://doi.org/10.1590/S0100-29452013000400017>

- Greenway H, Munns R. Mechanisms of salt tolerance in nonhalophytes. *Annu Rev Plant Physiol.* 1980;31:149–190. Doi: <https://doi.org/10.1146/annurev.pp.31.060180.001053>
- Hoagland DR, Arnon DI. The water-culture method for growing plants without soil. *Circ Calif Agric Exp Stn.* 1950;347:1–32.
- Hodson MJ, White PJ, Mead A, Broadley MR. Phylogenetic variation in the silicon composition of plants. *Ann Bot.* 2005;96(6):1027–1046. Doi: <https://doi.org/10.1093/aob/mci255>
- Holzschuh MJ, Bohnen H, Anghinoni I, Pizzolato TM, Carmona FDC, Carlos FS. Absorção de nutrientes e crescimento do arroz com suprimento combinado de amônio e nitrato. *Rev Bras Cienc Solo.* 2011;35(4):1357–1366. Doi: <https://doi.org/10.1590/S0100-06832011000400030>
- Horchani F, Hajri R, Aschi-Smiti S. Effect of ammonium or nitrate nutrition on photosynthesis, growth, and nitrogen assimilation in tomato plants. *J Plant Nutr Soil Sci.* 2010;173(4):610–617. Doi: <https://doi.org/10.1002/jpln.201000055>
- Jampeetong A, Brix H, Kantawanichkul S. Response of *Salvinia cucullata* to high NH_4^+ concentrations at laboratory scales. *Ecotoxicol Environ Saf.* 2012;79:69–74. Doi: <https://doi.org/10.1016/j.ecoenv.2011.12.003>
- Kraska JE, Breitenbeck GA. Simple, robust method for quantifying silicon in plant tissue. *Commun Soil Sci Plant Anal.* 2010;41(17):2075–2085. Doi: <https://doi.org/10.1080/00103624.2010.498537>
- Lawlor DW. Carbon and nitrogen assimilation in relation to yield: mechanisms are the key to understanding production systems. *J Exp Bot.* 2002;53(370):773–787. Doi: <https://doi.org/10.1093/jexbot/53.370.773>
- Li B, Li G, Kronzucker HJ, Baluška F, Shi W. Ammonium stress in *Arabidopsis*: signaling, genetic loci, and physiological targets. *Trends Plant Sci.* 2014;19(2):107–114. Doi: <https://doi.org/10.1016/j.tplants.2013.09.004>
- Liang Y, Si J, Römheld V. Silicon uptake and transport is an active process in *Cucumis sativus*. *New Phytol.* 2005;167(3):797–804. Doi: <https://doi.org/10.1111/j.1469-8137.2005.01463.x>
- Liang Y, Sun W, Zhu Y-G, Christie P. Mechanisms of silicon-mediated alleviation of abiotic stresses in higher plants: A review. *Environ Pollut.* 2007;147(2):422–428. Doi: <https://doi.org/10.1016/j.envpol.2006.06.008>
- Malavolta E, Vitti GC, Oliveira SA. Avaliação do estado nutricional das plantas: princípios e aplicações. 2nd ed. Piracicaba: POTAFOS; 1997. 319 p.
- Marschner P. Mineral nutrition of higher plants. 3 ed. London: Academic Press Elsevier; 2012. 651 p.
- Mauad M, Grassi Filho H, Crusciol CAC, Corrêa JC. Teores de silício no solo e na planta de arroz de terras altas com diferentes doses de adubação silicatada e nitrogenada. *Rev Bras Cienc Solo.* 2003;27(5):867–873. Doi: <https://doi.org/10.1590/S0100-06832003000500011>
- Mendoza-Villarreal R, Valdez-Aguilar LA, Sandoval-Rangel A, Robledo-Torres V, Benavides-Mendoza A. Tolerance of *Lisianthus* to high ammonium levels in rockwool culture. *J Plant Nutr.* 2015;38(1):73–82. Doi: <https://doi.org/10.1080/01904167.2014.920379>
- Mitani N, Ma JF. Uptake system of silicon in different plant species. *J Exp Bot.* 2005;56(414):1255–1261. Doi: <https://doi.org/10.1093/jxb/eri121>
- Nasraoui-Hajaji A, Gouia H. Photosynthesis sensitivity to NH_4^+ -N change with nitrogen fertilizer type. *Plant, Soil Environ.* 2014;60(6):274–279. Doi: <https://doi.org/10.17221/7418-PSE>
- Neto JFB, Pereira WE, Cavalcanti LF, Araújo RDC, Lacerda JS. Produtividade e qualidade de frutos de mamoeiro ‘Sunrise solo’ em função de doses de nitrogênio e boro. *Semin Cienc Agrárias.* 2011;32(1):69–80. Doi: <https://doi.org/10.5433/1679-0359.2011v32n1p69>
- Viciedo DO, de Mello Prado R, Lizcano Toledo RL, Santos LCND, Hurtado AC, Nedd LLT, *et al.* Silicon supplementation alleviates ammonium toxicity in sugar beet (*Beta vulgaris* L.). *J Soil Sci Plant Nutr.* 2019;19(2):413–419. Doi: <https://doi.org/10.1007/s42729-019-00043-w>
- Olivera Viciedo D, de Mello Prado R, Lizcano Toledo R, Nascimento dos Santos LC, Peña Calzada K. Response of radish seedlings (*Raphanus sativus* L.) to different concentrations of ammoniacal nitrogen in absence and presence of silicon. *Agron Colomb.* 2017;35(2):198–204. Doi: <https://doi.org/10.15446/agron.colomb.v35n2.62772>
- Prado RM. Nutrição de Plantas. 2nd ed. São Paulo, Brazil: UNESP; 2020.
- Rogato A, D’Apuzzo E, Barbulova A, Omrane S, Parlati A, Carfagna S, *et al.* Characterization of a developmental root response caused by external ammonium supply in *Lotus japonicus*. *Plant Physiol.* 2010;154(2):784–795. Doi: <https://doi.org/10.1104/pp.110.160309>
- Silva PCC, Lima do Couto J, Santos A. Absorção dos íons amônio e nitrato e seus efeitos no desenvolvimento do girassol em solução nutritiva. *Rev Biol Cienc Terra.* 2010;10(2):97–104.
- Su S, Zhou Y, Qin JG, Wang W, Yao W, Song L. Physiological responses of *Egeria densa* to high ammonium concentration and nitrogen deficiency. *Chemosphere.* 2012;86(5):538–545. Doi: <https://doi.org/10.1016/j.chemosphere.2011.10.036>
- Szczerba MW, Britto DT, Balkos KD, Kronzucker HJ. Alleviation of rapid, futile ammonium cycling at the plasma membrane by potassium reveals K^+ -sensitive and insensitive components of NH_4^+ transport. *J Exp Bot.* 2008;59(2):303–313. Doi: <https://doi.org/10.1093/jxb/ern309>
- Ten Hoopen F, Cuin TA, Pidas P, Hegelund JN, Shabala S, Schjoerring JK, *et al.* Competition between uptake of ammonium and potassium in barley and *Arabidopsis* roots: molecular mechanisms and physiological consequences. *J Exp Bot.* 2010;61(9):2303–2315. Doi: <https://doi.org/10.1093/jxb/erq057>

Von Wieren N, Lauter F-R, Ninnemann O, Gillissen B, Walch-Liu P, Engels C, *et al.* Differential regulation of three functional ammonium transporter genes by nitrogen in root hairs and by light in leaves of tomato. *Plant J.* 2000;21(2):167-175. Doi: <https://doi.org/10.1046/j.1365-313x.2000.00665.x>

Wang C, Zhang SH, Wang PF, Li W, Lu J. Effects of ammonium on the antioxidative response in *Hydrilla verticillata* (L.f.) Royle plants. *Ecotoxicol Environ Saf.* 2010;73(2):189-195. Doi: <https://doi.org/10.1016/J.ECOENV.2009.08.012>