Silicon Mitigates Nutritional Stress of Nitrogen, Phosphorus, and Calcium Deficiency in Two Forages Plants

William Bruno Silva Araújo

Federal Institute of Education, Science and Technology of Pará (IFPA) - Rural Campus of Marabá

Gelza Carliane Marques Teixeira (✉ gelzacarliane@hotmail.com)

São Paulo State University (Unesp)

Renato de Mello Prado

São Paulo State University (Unesp)

Antonio Márcio Souza Rocha

São Paulo State University (Unesp)

Research Article

Keywords: *Urochloa brizantha* cv. Marandu, *Megathyrsus maximum* cv. Massai, nutritive solution, plant nutrition, nutritional efficiency

Posted Date: December 10th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-1132722/v1>

License: This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Version of Record: A version of this preprint was published at Scientific Reports on April 22nd, 2022. See the published version at <https://doi.org/10.1038/s41598-022-10615-z>.

Abstract

Forages are one of the most cultivated crops in the world. However nutritional deficiency is common, specifically in N, P and Ca in many forages growing regions. Silicon (Si) can attenuate the stress caused by nutritional deficiency, but studies on the effects of Si supply on in forages plants are still scarce. This research was carried out to evaluate whether the Si supply can mitigate the effects of N, P and Ca deficiencies of two forages and the physiological and nutritional mechanisms involved. Two experiments were carried out with two forage species (*Urochloa brizantha* cv. Marandu and *Megathyrsus maximum* cv. Massai). Was used nutrient solution under balanced nutrition conditions and nutritional stress due to the lack of N, P and Ca combined with the -Si and +Si. The deficiencies of N, P and Ca in both forages cultivation caused damage to physiological and nutritional variables, hence decreasing the plant dry matter. However, in both species forages the addition of Si to the nutrient solution decreased the extravasation of cellular electrolytes and increased the content of phenolic compounds, the green color index, the quantum efficiency of photosystem II, the efficiencies of use of N, P and Ca and the production of shoot dry mass. The beneficial effects of Si were evidenced in stressed and non-stressed plants. The research emphasized the advantage of using Si for the growth of *U. brizantha* and *M. maximum* under N, P and Ca deficiency, contributing to their sustainable cultivation.

1. Introduction

Pastures occupy a vast cultivated area worldwide. This is because almost all beef production comes from animals fed only pasture due to its low cost of production¹. However, pasture cultivation often takes place in low fertility soils and under inadequate nutritional management². This fact is worrisome, as nutritional deficiency has a global impact on crop production and increases the seasonality of forage production³. In this sense, the nutrient uptake of plants depends on soil nutrient availability, and lack of nutrients can affect essential components of plant metabolism⁴. Moreover, grazing on nutrient-deficient forage affects the performance of animals and can cause nutritional disorders.

A strategy to mitigate the effects of nutritional deficiency in forages is the use of silicon (Si). This element can increase yield in Poaceae plants as they are Si accumulators⁵. Soil Si availability decreases in pasture areas due to degradation at different levels,⁶ being aggravated by Si export via grazing without replacement. The main mechanisms of action of Si correlate with oxidative damage reduction, thus increasing photosynthetic rate and nutrient uptake.⁷ This benefit of Si in the antioxidant defense system of plants has to do with its effect on the biosynthesis of phenolic compounds 8, ⁹.

The literature also indicates that Si can bind calcium-like compounds to organic compounds in the cell wall; thus, Si can occupy calcium-binding sites, further minimizing the effects of Si deficiency¹⁰. Other studies added that Si can replace part of the carbon in organic structures of the cell wall⁷ with low energy cost,¹¹ enhancing nutrient uptake and nutrient use efficiency.

However, most of these beneficial effects have been recorded in plants under biotic stress¹². Studies addressing abiotic stresses refer to damage by salinity¹³, toxicity of chemical elements¹⁴, and water conditions^{15,16,17}. Regarding nutritional disorders, studies have focused on the benefit of Si in relieving nitrogen (N) deficiency in rice¹⁸, potassium (K) deficiency in sorghum¹⁹ and maize²⁰, magnesium (Mg) deficiency in maize,²¹ calcium (Ca) deficiency in cabbage²² and rocket²³, phosphorus (P) deficiency in sorghum²⁴, wheat²⁵, and cucumber²⁶, and manganese (Mn) deficiency in sugarcane²⁷.

The beneficial effects of Si are more evident in plants with high accumulation of this element, such as those of the Poaceae family, including forages²⁸. Notwithstanding, studies evaluating the effect of Si on the mitigation of abiotic stresses in forage plants are still limited. One study indicates a positive effect of this element on the alleviation of water deficit damage in *Brachiaria* 'Mavuno'²⁹. Another study addresses nutritional deficiency in *Panicum maximum* and in the hybrid Ipyporã (*B. ruziziensis* × *B. brizantha*)³⁰. Considering only the genera *Urochloa* and *Megathyrus*, as far as we know there is no previous report on the use of Si to alleviate nutritional deficiencies. Despite that, these forage species adapt to various environmental conditions and are widely cultivated worldwide³¹.

These forages present low yield in tropical regions, especially due to deficiency of macronutrients³² such as N, P, and Ca. It is not yet known whether Si could contribute to mitigate nutritional deficiency in these species, or the mechanisms of action by which it would occur. However, this information would be important to increase the sustainability of tropical forage production systems, as these species are mostly grown in low fertility soils.

In this context, the hypothesis arises that Si can alleviate the effects of N, P, and Ca deficiency in forage crops. Thus, this research assesses whether Si application can alleviate the effects of N, P, and Ca deficiency in two forages, as well as the physiological and nutritional mechanisms involved.

2 Material And Methods

2.1 Experimental conditions

Two experiments were carried out simultaneously in a greenhouse located at UNESP, Câmpus de Jaboticabal, from January to June 2019. Data on relative air humidity and temperature maximum and minimum inside the greenhouse were recorded daily with the aid of a thermohygrometer (U23-001, Sigma Sensors, Brazil). There was a variation in the relative air humidity ($60 \pm 15\%$), maximum temperature ($40 \pm 10^\circ\text{C}$), and minimum temperature ($32 \pm 10^\circ\text{C}$).

We used two forage species *U. brizantha* cv. Marandu (Experiment 1) and *M. maximum* cv. Massai (Experiment 2), obtained from the Brazilian Agricultural Research Corporation of the Ministry of Agriculture, Livestock and Food Supply. All plant studies were carried out in accordance with relevant institutional, national, or international guidelines and regulation. Our research was not conducted with

endangered species and was conducted in accordance with the is in accordance with the Declaration of IUCN Policy on Research Involving Endangered Species.

Sowing was carried out in polypropylene trays containing vermiculite as a substrate. The plants were kept in the trays for 20 days, receiving only distilled water. After this period, the seedlings were selected, washed, and transplanted to plastic pots with a volume of 1.7 dm³, filled with previously washed sand of medium size. After transplanting the plants, the application of the complete nutrient solution was carried out as indicated by Hoagland and Arnon³³, with ionic strength of 25%, over 4 days. Then, the nutrient solution was applied according to the treatments with ionic strength adjusted to 50% until the end of the experiment. The variation of the ionic strength was performed to avoid salinity stress to the plants.

The nutrient solutions were prepared with distilled and deionized water. Was performed a change in the iron source from Fe-EDTA to Fe-EDDHMA using double the dose as recommended³⁴. The pH value was maintained at 5.5±0.2 and monitored daily with a digital pH meter (ICEL PH-1500); when necessary, it was adjusted with HCl or NaOH solution.

2.2 Experimental design and treatments

The experiment was carried out in a 4 × 2 factorial scheme. The treatments consisted of control (complete solution - CS); solution deficient of N (-N), P (-P) and Ca (-Ca); combined without (-Si) and with of Si (+Si). Treatments were arranged in experimental design was completely randomised, with six replicates.

The nutrient solution for the treatments was prepared according to Hoagland and Arnon³³. For treatments with -N, a deficiency equivalent to 50% of the maximum dry mass production was simulated³⁵. Nitrogen was supplied in the form of ammonium nitrate (NH₄NO₃) at a concentration of 3.0 mmol L⁻¹ for three days, followed by 4.5 mmol L⁻¹ for the same period and 6.0 mmol L⁻¹ until the end of the experiment.

In treatments with -P, this nutrient was initially omitted from the nutrient solution and, after 30 days, 0.05 mmol L⁻¹ was added until the end of the experiment. For treatments with -Ca, the nutrient was omitted from the beginning to until the end of the experiment.

The supply of Si was started after 5 days of emergence, applying only to the +Si treatment. The Si source was sodium silicate stabilised with sorbitol (94.2 g L⁻¹ of Si and 60 g L⁻¹ of Na, pH 12.6), at a concentration of 2.0 mmol L⁻¹, supplied via nutrient solution (via root). Silicon was added to the nutrient solution, and the pH value was adjusted to 5.5±0.2 with Hydrochloric acid (HCl) or Sodium hydroxide (NaOH) solutions, and it was immediately supplied to the plants. The additional application of 49.8 mg L⁻¹ of NaCl was performed in the treatments -Si to balance the Na among treatments.

Once a week, the substrate was washed to avoid salinization. the deionized water was applied in the substrate until drainage, and the nutrient solution was applied again after 2 h. After 45 days after

transplanting, was a standardization cut was performed in the forages at a height of 5 cm from the substrate.

2.3 Analyses

After 90 days of transplantation, with the expression of classic signs of nutrient deficiency, were evaluated of the electrolyte leakage index, green color index, phenolic compounds, and quantum yield of photosystem II. These evaluations were carried out on the second fully expanded leaf in the treatments with -N and -P; and on the first fully expanded leaf for -Ca. In the CS plants, data were also collected for these two leaves.

2.3.1 Green color index

The green color index was measured with the aid of Opti-sciences® equipment - CCM-200, from three readings carried out on the sheets fully expanded according to the treatment.

2.3.2 Quantum yield of photosystem II (Fv/Fm)

Quantum yield of photosystem II (Fv/Fm) was measured in the morning (7 to 8 a.m.), using a fluorometer device (Opti-sciences® - Os30P +). For this measurement, the sampled region was placed in the dark for adaptation at least 30 min before the excitation of the red-light pulse of 1 s³⁶. Readings were taken in the middle third of the leaf avoiding the midrib. Was used to the second fully expanded leaf in the treatments with -N and -P, on the first fully expanded leaf for -Ca, and of both leaves in the CS.

2.3.3 Electrolyte leakage index

For determining the electrolyte leakage index, ten leaf discs (6 mm) were removed from the middle third of the second fully expanded leaf in the treatments with -N and -P, on the first completely expanded leaf for -Ca, and of both leaves in the CS.

The discs were packed in a beaker with 20 mL of deionized water at ambient temperature for 2 h. After this period, the initial electrical conductivity reading (EC₁) was performed using a manual conductivity meter (TDS-3). Afterward, samples were taken to autoclave for 20 min at a temperature of 121°C. After cooling, a new conductivity reading was performed to determine the final conductivity (EC₂). To estimate the electrolyte leakage index, the following formula was used: $EC_1/EC_2 \times 100^{37}$.

2.3.4 Phenolic compounds

Quantification of total phenols content was performed in the middle portion of the second fully expanded leaf using the method as described by Singleton et al.³⁸. For such, samples of fresh leaves were emerged in concentrated methanol in a water bath at 25°C. After extraction, a colorimetric reaction of total phenols was induced with the 2 N Folin-Ciocalteu reagent, allowing reacting for three minutes, and 20% sodium carbonate, allowing reacting for 2 h. In the end, the absorbance was read on a spectrophotometer at a

wavelength of 765 nm, while the content was determined using a standard curve with gallic acid, expressed as Gallic acid equivalent (GAE) 100 g⁻¹.

2.3.5 Plant length and number of tillers

At 55 days after the forage uniformity cut, he measured the length of the plants from the base to the top of the plant using a measuring tape. On this day, all forage tillers were counted.

2.3.6 Dry matter

At 55 days after the uniform cut the plants were harvested then washed in running water with a detergent solution (0.1% v/v), HCl solution (0.3% v v) and twice in deionized water. Subsequently, the plant material was oven-dried with forced air circulation (65 ± 5°C) until it reached constant mass. Then, the shoot dry matter of the forages was obtained.

2.3.7 Si accumulation

The Si content was determined in samples obtained from shoots and roots of forages. For such, 0.1 g of dry and ground material, which were added to 50 mL polyethylene tubes. The samples were moistened with 2 mL of hydrogen peroxide (H₂O₂), the tube was placed in an oven at 95°C. After 30 min, the tubes were removed, and 4 mL of 50% NaOH were added to warm samples. The sample tubes were then gently vortexed and returned to the oven (95°C) for four hours according to the methodology described in Kraska et al.³⁹. The Si concentration was determined by colorimetry using 1 mL extract plus 19 mL of water, 1 mL of HCl (1:1), and 2 mL ammonium molybdate. After 5 min, 2 mL oxalic acid were added. The reading performed by a spectrophotometer at 410 nm as described in Korndörfer et al.⁴⁰.

2.3.8 N, P and Ca accumulation and use efficiencies

The N content was determined by adding concentrated sulfuric acid to samples of previously dried and ground plant material, followed by distillation and titration with sulfuric acid⁴¹. The levels of P and Ca were determined by the digestion of samples of plant material, using a digestive mixture of perchloric and nitric acid (1:2), with readings of Ca performed in spectrophotometry atomic absorption with air-acetylene flame and P readings through spectrophotometry⁴¹.

Based on the content and shoot dry matter, the accumulation of each nutrient in the shoot of the forages was obtained to be used in the calculation of the N, P, and Ca use efficiencies, according to the equation: (shoot dry matter)² / nutrient accumulation in shoot⁴².

2.4 Statistical analysis

The data obtained were submitted to analysis of variance by the F test. If significant ($p < 0.01$) and for differentiation between treatments, Tukey's means comparison test ($p < 0.05$) was performed using the SAS statistical software (Cary, NC, USA).

3 Results

3.1 Experiment 1 – *U. brizantha* cv. Marandu

The accumulation of Si in the shoot decreased in plants grown under deficiencies in N, P and Ca compared to the CS. However, the addition of Si to forage plants without and with nutritional deficiency studied in relation to treatments without Si, increased the accumulation of Si in the shoot of the plant (Fig. 1a).

The accumulation of Si in the plant roots decreased with deficiencies in N, P, independent of Si, and with Ca deficiency in the absence of Si in comparison with the CS. The addition of Si in plants under CS, and without addition of P and Ca, compared to the control treatment (without Si) increased the accumulation of Si in the root and shoot of the forages (Fig. 1b). Accumulation of Si in the absence of N addition did not differ in relation to the presence or absence of Si.

Plants that received a solution deficient in P and Ca, independent of Si, compared to plants under N deficiency only in the absence of Si, showed an increase in the electrolyte leakage index. The supply of Si in all treatments (-N, -P, -Ca and CS) compared to treatments that did not receive Si, decreased the electrolyte leakage index (Fig. 2a).

The levels of phenolic compounds in plants grown in CS and in solutions deficient of N and P were similar. But the content of these compounds increased in the solution deficient in Ca, independent of Si addition. Phenol's content increased in the aerial part of the forage because of the addition of Si to both solutions (Fig. 2b).

The green color index decreased in forage cultivated with a solution deficient in N, P and Ca, compared to the CS, independent of Si. However, this index increased due to the increase of Si in plants deficient in N, P and Ca (Fig. 3a).

The forage grown with nutrient-deficient solution in relation to forage grown with CS, independent of Si, have decreased of the quantum yield of photosystem II (Fv/Fm). However, with the addition of Si to the nutrient solution, fluorescence quantum yield increased (Fig. 3b).

The N, P and Ca use efficiencies decreased in forage grown with solutions deficient in these nutrients, regardless of the +Si or -Si. However, use efficiencies increased in plants deficient in these nutrients because of the addition of Si (Fig. 4a, 4b and 4c).

Plant length decreased when plants were cultivated in solution deficient in N, P and Ca in relation to the CS, independent of Si. However, the addition of Si resulted in increased plant length, irrespective of the nutrient solution (Fig. 5a).

Tiller number of plants cultivated in solution deficient of N and P decreased in relation to the control group, independent of Si. However, under Ca deficiency, the number of tillers was higher than that of plants grown in CS. The addition of Si to the control and to the solutions deficient in N and P increased the number of tillers (Fig. 5b).

The shoot dry matter production was impaired in forage cultivated in solution deficient of N, P and Ca, irrespective of Si addition. In contrast, the addition of Si to the control group and the group deficient in N, P and Ca increased the shoot dry matter production (Fig. 5c).

3.2 Experiment 2 – *M. maximum* cv Massai

The accumulation of Si in the shoot decreased under deficiencies of N, P and Ca compared to the CS, in the presence of Si. The addition of Si resulted in increased Si accumulation in the shoot (Fig. 1c).

The accumulation of Si in the root was lower in plants cultivated in solution deficient of N and P and increased when Ca was deficient, independent of Si addition. The addition of Si to the control and to the solutions deficient in N, P and Ca increased accumulation of Si in the roots (Fig. 1d).

The electrolyte leakage index increased in plants deficient in N, P and Ca, irrespective of Si addition. However, the addition of Si decreased the electrolyte leakage index in all treatments regardless of if grown with CS solution or with -N, -P, and -Ca solution (Fig. 2c).

The levels of phenolic compounds decreased with Ca deficiency, independent of Si, and with N deficiency only in the presence of Si. Under P deficiency, the phenols content in the forage increased in relation to those grown in CS solution, albeit only in the absence of Si. The addition of Si increased the plant phenols content, regardless of whether it was grown in a control solution or under -N, -P and -Ca (Fig. 2d).

The green color index was lower when plants were grown under -N, -P and -Ca, independent of Si. However, the addition of Si increased the green color index in the forage grown in the control and in solutions lacking N, P and Ca (Fig. 3c).

The fluorescence quantum yield (Fv/Fm) was lower when plants were grown under -P and -Ca, independent of Si, and under -N in the absence of Si. The addition of Si increased the quantum yield of forage grown under -N, -P and -Ca in relation to the -Si but did not change this parameter for plants grown in the CS (Fig. 3d).

The N use efficiency was lower under nutrient deficiency, independent of Si. However, it increased when plants were deficient in N and Si was added (Fig. 4d). The P use efficiency was lower under P deficiency, both in the +Si or -Si in the nutrient solution. However, it increased when Si was added (Fig. 4e). The Ca use efficiency was higher when plants were grown without Ca, independent of Si. The addition of Si increased this parameter (Fig. 4f).

Plant length decreased in all treatments deficient in N, P and Ca compared to treatments receiving CS solution, independent of Si. However, the addition of Si had no positive impact on plant length (Fig. 5d). The number of tillers decreased under -N, -P and -Ca, regardless of Si supply. However, the addition of Si to the nutrient solution increased tiller number only when N and P were lacking (Fig. 5e).

The shoot dry matter decreased when cultivated under -N, -P and -Ca compared to the CS, regardless of the +Si or -Si. However, this parameter increased with the addition of Si to all treatments (Fig. 5f).

4 Discussion

4.1 Silicon improves the performance of forage plants without nutritional deficiency

Silicon addition in the complete nutrient solution favored shoot dry matter production in both forages under all conditions. There is little evidence of the beneficial effects of Si on plants grown without specific nutritional disorders. Nevertheless, some reports indicate the positive effects of Si on crops under biotic¹² or abiotic stresses^{19,15}. In the present study, the beneficial effect of Si on dry matter production was evident even in plants grown without nutritional deficiency. This Si effect occurred due to its positive effects on antioxidant capacity, evidenced by increased production of phenolic compounds. This in turn reduced the electrolyte leakage index, increasing green color index, fluorescence quantum yield, and ultimately N, P, and Ca use efficiencies.

This effect of Si might also be because this element could decrease the demand for other compounds of the cell wall, such as lignin, which demands more energy¹¹. Silicon can replace part of the carbon in the formation of some low energy cost structural compounds⁷, especially in the cell wall²⁸. In grasses, the element concentrates mostly in leaf cell walls¹¹, which may improve nutrient use efficiency, thus favoring biomass production. Previous reports show this effect for sugarcane⁴³ and *Brachiaria* 'Mavuno'²⁹. However, confirmation of this hypothesis depends on specific studies on the cell walls of *U. brizantha* cv. Marandu and *M. maximum* cv Massai.

The beneficial effect even on plants without nutritional stress is due to the high accumulation of Si, above all, in the shoots of both forage species under study, showing their great capacity to absorb and accumulate this element⁵. This result indicates that Si can be used for the cultivation of forage species without nutritional deficiency to increase the sustainability of the cultivation of these species through a better management of macronutrient supply. Other studies show similar results for sugarcane²⁷ and forage plants (*P. maximum* and the hybrid Ipyorã (*B. ruziziensis* × *B. brizantha*))⁴⁴.

4.2 Silicon mitigates nutritional N deficiency in forage plants

Plants grown in N-deficient nutrient solution showed impaired growth. This is due to the participation of N in the constitution of enzymes and root cell membrane transporters⁴⁵. Nitrogen is involved in the active absorption of nutrients; thus, its deficiency affects this process negatively. Moreover, the low leaf N content directly interfered with pigments, as N is a structural nutrient of the chlorophyll molecule^{46, 32}.

This was evidenced by the decrease in leaf green color index. Loss of pigmentation in leaves under N deficiency also has to do with the redistribution of this element due to proteolysis of the enzyme Rubisco and other chloroplast proteins, releasing the N present in these compounds to meet the demand for new organs⁴⁷.

This decrease in nitrogen reduced the photosynthetic rate, as photosynthetic pigments play a key role in transferring excitation energy to photosystems⁴⁸. The result was oxidative stress, evidenced by increased cellular electrolyte leakage. The reduction of phenolic compounds (which can act as nonenzymatic antioxidants) further aggravated stress⁴⁹. These effects impaired plant growth and dry matter production.

On the other hand, Si supply physiologically improved plants under nutritional deficiencies. This was evidenced by the increase in phenolic compounds, green color index, and fluorescence quantum yield (Fv/Fm). Other authors have reported similar results for barley⁹ and ryegrass^{8, 19}. In these studies, Si increased phenol production, thus improving the antioxidant system and increasing chlorophyll content as well as photosystem II efficiency.

Nitrogen-deficient plants that received Si showed better physiological conditions, with increased N and Ca use efficiencies and beneficial effects on plant growth and shoot dry matter production. Nutritional deficiencies can limit plant growth by causing imbalances and reducing nutrient use efficiency, which in turn decreases biomass production⁴⁵. Thus, by improving nutritional efficiency, Si reduces the damage caused by N deficiency in these forage crops.

In this sense, the benefits of Si in mitigating the effects of N deficiency in the forages *U. brizantha* cv. Marandu and *M. maximum* cv Massai are unprecedented, like the results of a study investigating other grasses such as rice¹⁸.

Silicon application improved the performance of the two forage species under N deficiency. This suggests that the use of Si is a good strategy to enhance N fertilization. This strategy has high sustainability since it preserves N, whose production depends on natural gas, a nonrenewable source⁵⁰. These results are important because the occurrence of low levels of N is common in forage cultivation areas³² due to the degradation scenario that reduces organic matter, an important source of N 50, ¹.

4.3 Silicon mitigates nutritional P deficiency in forage plants

Plants grown in P-deficient nutrient solution showed impairments in all variables under study. This is because P plays an important role in the energy metabolism of plants and in the formation of ATP and ADP.⁵¹ Low P levels in the plant affect plant growth and development due to lower formation of new cells and lower cell elongation.⁵² Moreover, P correlates with metabolic activities, and its low supply directly affects gas exchange, photosynthetic rate, and the activity of ribulose 1,5-bisphosphate carboxylase (Rubisco)⁵³.

These effects increase the production of reactive oxygen species that degrade the cell membrane due to lipid oxidation⁵⁴. This can be seen from the increased rate of electrolyte leakage and the decreased synthesis of phenolic compounds, as indicated by Ma et al.⁵⁵ in wheat plants. The present study

demonstrated this situation for the first time in the forage plants *U. brizantha* cv. Marandu and *M. maximum* cv Massai. However, further studies have demonstrated it in other grasses such as sorghum²⁴, cucumber⁵⁶, snap bean⁵⁷, and quinoa⁵⁸.

Silicon addition alleviated the damage of P deficiency, a fact not yet described in the literature for these species. This finding can be explained by Si-mediated improvements in the antioxidant system of these plants through the influence of this element on the metabolism of phenolic compounds. The plasma membrane was thus preserved, with lower electrolyte leakage index, which increased the green color index. This suggests a higher chlorophyll content⁵⁹, favoring photosynthetic efficiency, which results in energy (ATP) that is important for P transporters⁶⁰.

Silicon can also increase the gene expression of P transporters, and this can favour P uptake by plants deficient in this nutrient²⁵. Some reports have already described this effect for K, in which Si improved its absorption by the plant from the activation of H-ATPase⁶¹. The Si-mediated activation of nutrient transporting enzymes may explain the increase in P use efficiency in both forage species under study. The literature shows similar results for wheat²⁵ and sorghum²⁴ under P.

4.4 Silicon mitigates nutritional Ca deficiency in forage plants

Cultivation under Ca deficiency reduced plant growth, green color index, and fluorescence quantum yield. This fact was due to a lower expansion and lack of rigidity of the cell wall, which is governed by Ca²⁺ bonds to pectates⁶². The Ca within the cytosol can also act as a signal for several enzymes related to photosynthesis and as a secondary messenger in both biotic and abiotic stresses⁶², contributing to the decrease in dry matter accumulation. In addition, for both forages under study, Ca-deficient plants that did not receive Si in the nutrient solution presented a decrease in Ca use efficiency, which decreased dry matter production.

Notwithstanding, these negative impacts were mitigated with the addition of Si. This beneficial effect of silicon on plants was evidenced by its accumulation in the shoots of Ca-deficient plants, which increased the production of phenolic compounds. Other authors have also reported this effect for ryegrass⁸, barley⁹, and rocket plants²³. The increase in phenols possibly occurred because Si structurally improves cell wall components, in which Ca is involved. Silicon forms complexes with structural cell polymers such as pectin and callose⁶³, crosslinking with lignins and carbohydrates via associations with phenolic acids or aromatic rings¹⁰. This improves the structuring of the cell wall and decreases the extravasation of oxidative agents.

The antioxidant action of Si contributed to the increase in green color index and leaf fluorescence quantum yield. Therefore, it reduced the damage caused to cell wall integrity, as can be seen from the lower electrolyte leakage index. Silicon addition increased Ca use efficiency and shoot dry matter production. Other studies have already demonstrated the positive effects of Si on the attenuation of Ca

deficiency in rocket,²³ cabbage²², and *P. maximum* plants³⁰, as well as in the hybrid Ipyporã (*B. ruziziensis* × *B. brizantha*)³⁰. However, this is the first evidence that it also occurs in the forages *U. brizantha* cv. Marandu and *M. maximum* cv Massai.

The unprecedented information obtained about the benefits of Si in alleviating nutritional deficiency in the forages under study indicates the importance of this beneficial element for the sustainability of the cultivation of these species, as they are usually grown in low-fertility tropical soils. It is now important to expand these studies to other forage species and other nutrients, with the aim to increase knowledge about the benefits of Si in the physiology and nutrition of forage plants.

5 Conclusion

Silicon mitigates the effects of N, P and Ca deficiency, improves antioxidant activity and photosynthesis rates as well as N, P and Ca use efficiencies and dry matter production of forage plants. The research emphasized the advantage of using Si for the growth of *U. brizantha* and *M. maximum* under N, P and Ca deficiency, contributing to their sustainable cultivation.

Declarations

Funding sources

This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001.

Conflict of Interest

The authors declare that they have no conflict of interest.

References

1. Pereira, O. J. R., Ferreira, L. G., Pinto, F. & Baumgarten, L. Assessing pasture degradation in the Brazilian Cerrado based on the analysis of MODIS NDVI time-series. *Remote Sensing* **10**, (2018).
2. Vale, P. *et al.* The expansion of intensive beef farming to the Brazilian Amazon. *Global Environmental Change* **57**, 101922 (2019).
3. Sbrissia, A. F., Schmitt, D., Duchini, P. G. & Da Silva, S. C. Unravelling the relationship between a seasonal environment and the dynamics of forage growth in grazed swards. *Journal of Agronomy and Crop Science* **206**, 630–639 (2020).
4. Grusak, M. A., Broadley, M. R. & White, P. J. Plant macro- and micronutrient minerals. *eLS* 1–6 (2016) doi:10.1002/9780470015902.a0001306.pub2.
5. Katz, O., Puppe, D., Kaczorek, D., Prakash, N. B. & Schaller, J. Silicon in the soil–plant continuum: Intricate feedback mechanisms within ecosystems. *Plants* **10**, 1–36 (2021).

6. Yang, S. *et al.* Impact of grassland degradation on the distribution and bioavailability of soil silicon: Implications for the Si cycle in grasslands. *Science of the Total Environment* **657**, 811–818 (2019).
7. Cooke, J. & Leishman, M. R. Consistent alleviation of abiotic stress with silicon addition: a meta-analysis. *Functional Ecology* **30**, 1340–1357 (2016).
8. Ribera-Fonseca, A., Rumpel, C., Mora, M. D. L. L., Nikolic, M. & Cartes, P. Sodium silicate and calcium silicate differentially affect silicon and aluminium uptake, antioxidant performance and phenolics metabolism of ryegrass in an acid Andisol. *Crop and Pasture Science* **69**, 205–215 (2018).
9. Vega, I. *et al.* Silicon improves the production of high antioxidant or structural phenolic compounds in barley cultivars under aluminum stress. *Agronomy* **9**, (2019).
10. Inanaga, S., Okasaka, A. & Tanaka, S. Does silicon exist in association with organic compounds in rice plant? *Soil Science and Plant Nutrition* **41**, 111–117 (1995).
11. Neu, S., Schaller, J. & Dudel, E. G. Silicon availability modifies nutrient use efficiency and content, C:N:P stoichiometry, and productivity of winter wheat (*Triticum aestivum* L.). *Scientific Reports* **7**, 3–10 (2017).
12. Tatagiba, S. D., DaMatta, F. M. & Rodrigues, F. A. Silicon partially preserves the photosynthetic performance of rice plants infected by *Monographella albescens*. *Annals of Applied Biology* **168**, (2016).
13. Hurtado, A. C. *et al.* Different methods of silicon application attenuate salt stress in sorghum and sunflower by modifying the antioxidative defense mechanism. *Ecotoxicology and Environmental Safety* **203**, (2020).
14. dos Santos, M. S. *et al.* Silicon nutrition mitigates the negative impacts of iron toxicity on rice photosynthesis and grain yield. *Ecotoxicology and Environmental Safety* **189**, (2020).
15. Teixeira, G. C. M., Mello Prado, R. & Rocha, A. M. S. Low absorption of silicon via foliar in comparison to root application has an immediate antioxidant effect in mitigating water deficit damage in sugarcane. *Journal of Agronomy and Crop Science* **00**, 1–10 (2021).
16. Teixeira, G. C. M., Prado, R. de M., Rocha, A. M. S. & Piccolo, M. de C. Root- and foliar-applied silicon modifies C: N: P ratio and increases the nutritional efficiency of pre-sprouted sugarcane seedlings under water deficit. *PLoS ONE* **15**, 1–24 (2020).
17. Teixeira, G. C. M., Mello Prado, R. de, Rocha, A. M. S., Silveira Sousa Junior, G. da & Gratão, P. L. Beneficial Effect of Silicon Applied Through Fertigation Attenuates Damage Caused by Water Deficit in Sugarcane. *Journal of Plant Growth Regulation* (2021) doi:10.1007/s00344-021-10510-3.
18. Deus, A. C. F., de Mello Prado, R., de Cássia Félix Alvarez, R., de Oliveira, R. L. L. & Felisberto, G. Role of silicon and salicylic acid in the mitigation of nitrogen deficiency stress in rice plants. *Silicon* **12**, 997–1005 (2020).
19. Chen, D. *et al.* Silicon moderated the K deficiency by improving the plant-water status in sorghum. *Scientific Reports* **6**, 1–14 (2016).
20. Sarah, M. M. dos S. *et al.* Silicon supplied via roots or leaves relieves potassium deficiency in maize plants. *Silicon* (2021) doi:10.1007/s12633-020-00908-1.

21. Hosseini, S. A., Rad, S. N., Ali, N. & Yvin, J. C. The ameliorative effect of silicon on maize plants grown in Mg-deficient conditions. *International Journal of Molecular Sciences* **20**, 969 (2019).
22. da Silva, D. L., de Mello Prado, R., Tenesaca, L. F. L., da Silva, J. L. F. & Mattiuz, B. H. Silicon attenuates calcium deficiency by increasing ascorbic acid content, growth and quality of cabbage leaves. *Scientific Reports* **11**, 1–9 (2021).
23. da Silva, D. L., Prado, R. de M., Tenesaca, L. F. L., da Silva, J. L. F. & Mattiuz, B. H. Silicon attenuates calcium deficiency in rocket plants by increasing the production of non-enzymatic antioxidants compounds. *Scientia Horticulturae* **285**, (2021).
24. Silva, J. L. F. da & Prado, R. de M. Elucidating the action mechanisms of silicon in the mitigation of phosphorus deficiency and enhancement of its response in sorghum plants. *Journal of Plant Nutrition* 1–11 (2021) doi:10.1080/01904167.2021.1918155.
25. Kostic, L., Nikolic, N., Bosnic, D., Samardzic, J. & Nikolic, M. Silicon increases phosphorus (P) uptake by wheat under low P acid soil conditions. *Plant and Soil* **419**, 447–455 (2017).
26. Campos, C. N. S. *et al.* Macronutrient deficiency in cucumber plants: impacts in nutrition, growth and symptoms. *Journal of Plant Nutrition* **44**, (2021).
27. Oliveira, K. S., de Mello Prado, R., Checchio, M. V. & Gratão, P. L. Silicon via nutrient solution modulates deficient and sufficient manganese sugar and energy cane antioxidant systems. *Scientific Reports* **11**, 1–14 (2021).
28. de Melo, S. P., Monteiro, F. A. & de Bona, F. D. Silicon distribution and accumulation in shoot tissue of the tropical forage grass *Brachiaria brizantha*. *Plant and Soil* **336**, 241–249 (2010).
29. Rocha, J. R., de Mello Prado, R., Teixeira, G. C. M. & de Oliveira Filho, A. S. B. Si fertigation attenuates water stress in forages by modifying carbon stoichiometry, favouring physiological aspects. *Journal of Agronomy and Crop Science* 1–13 (2021) doi:10.1111/jac.12479.
30. Buchelt, A. C. *et al.* Silicon Contribution Via Nutrient Solution in Forage Plants to Mitigate Nitrogen, Potassium, Calcium, Magnesium, and Sulfur Deficiency. *Journal of Soil Science and Plant Nutrition* **20**, (2020).
31. Monteiro, F. A. *et al.* Cultivo de *Brachiaria brizantha* Stapf. cv. Marandu em solução nutritiva com omissões de macronutrientes. *Scientia Agricola* **52**, 135–141 (1995).
32. Prado, R. de M. *Mineral nutrition of tropical plants*. (Springer International Publishing, 2021). doi:10.1007/978-3-030-71262-4.
33. Hoagland, D. R. & Arnon, D. I. *The water-culture method for growing plants without soil*. (The College of Agriculture University of California, 1950).
34. Cavalcante, V. S., Prado, R. M., Vasconcelos, R. L. & Campos, C. N. S. Iron concentrations in sugar cane (*Saccharum officinarum* L.) cultivated in nutrient solution. *Agrociencia* **50**, 867–875 (2016).
35. Lavres Junior, J. & Antonio Monteiro, F. Combinações de doses de nitrogênio e potássio para a produção e nutrição do capim-mombaça. *Boletim de Indústria Animal* **59**, 101–114 (2002).

36. Lichtenthaler, H. K., Buschmann, C. & Knapp, M. How to correctly determine the different chlorophyll fluorescence parameters and the chlorophyll fluorescence decrease ratio RFd of leaves with the PAM fluorometer. *Photosynthetica* **43**, 379–393 (2005).
37. Dionisio-Sese, M. L. & Tobita, S. Antioxidant responses of rice seedlings to salinity stress. *Plant Science* **135**, 1–9 (1998).
38. Singleton, V. L. & Rossi, J. A. Jr. Colorimetry to total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American Journal of Enology and Viniculture* **16**, 144–58 (1965).
39. Kraska, J. E. & Breitenbeck, G. A. Simple, robust method for quantifying silicon in plant tissue. *Communications in Soil Science and Plant Analysis* **41**, 2075–2085 (2010).
40. Korndörfer, G. H., Pereira, H. S. & Nolla, A. *Análise de silício no solo, planta e fertilizantes*. (UFU, 2004).
41. Bataglia, O. C., Furlani, A. M. C., Teixeira, J. P. F., Furlani, P. R. & Gallo, J. R. *Métodos de análise química de plantas*. (1983).
42. Siddiqi, M. Y. & Glass, A. D. M. Utilization index: A modified approach to the estimation and comparison of nutrient utilization efficiency in plants. *Journal of Plant Nutrition* **4**, 289–302 (1981).
43. Frazão, J. J., Prado, R. de M., de Souza Júnior, J. P. & Rossatto, D. R. Silicon changes C:N:P stoichiometry of sugarcane and its consequences for photosynthesis, biomass partitioning and plant growth. *Scientific Reports* **10**, 1–10 (2020).
44. Buchelt, A. C. *et al.* Silicon contribution via nutrient solution in forage plants to mitigate nitrogen, potassium, calcium, magnesium, and sulfur deficiency. *Journal of Soil Science and Plant Nutrition* **20**, 1532–1548 (2020).
45. Beshir, M. H., Tesfaye, B., Bueckert, R. & Tar'an, B. Pod quality of snap bean as affected by Nitrogen fixation, cultivar and climate zone under dryland agriculture. *African Journal of Agricultural Research* **10**, 3157–3169 (2015).
46. Takahashi, H., Kopriva, S., Giordano, M., Saito, K. & Hell, R. Sulfur assimilation in photosynthetic organisms: molecular functions and regulations of transporters and assimilatory enzymes. *Annual Review of Plant Biology* **62**, 157–184 (2011).
47. Feller, U., Anders, I. & Demirevska, K. Degradation of rubisco and other chloroplast proteins under abiotic stress. *General and Applied Plant Physiology GAPP* **34**, 5–18 (2008).
48. Havaux, M. Carotenoid oxidation products as stress signals in plants. *Plant Journal* **79**, 597–606 (2014).
49. Hiraga, S., Sasaki, K., Ito, H., Ohashi, Y. & Matsui, H. A large family of class III plant peroxidases. *Plant and Cell Physiology* **42**, 462–468 (2001).
50. Diacono, M. & Montemurro, F. Long-term effects of organic amendments on soil fertility. A review. *Agronomy for Sustainable Development* **30**, 401–422 (2010).
51. Lin, Z. H. *et al.* CO₂ assimilation, ribulose-1,5-bisphosphate carboxylase/ oxygenase, carbohydrates and photosynthetic electron transport probed by the JIP-test, of tea leaves in response to phosphorus

- supply. *BMC Plant Biology* **9**, 1–12 (2009).
52. Sun, L., Tian, J., Zhang, H. & Liao, H. Phytohormone regulation of root growth triggered by P deficiency or Al toxicity. *Journal of experimental botany* **67**, 3655–3664 (2016).
 53. Santos, K. R. *et al.* Typha domingensis Pers. growth responses to leaf anatomy and photosynthesis as influenced by phosphorus. *Aquatic Botany* **122**, 47–53 (2015).
 54. Fu, Y. Q., Yang, X. J. & Shen, H. The physiological mechanism of enhanced oxidizing capacity of rice (*Oryza sativa* L.) roots induced by phosphorus deficiency. *Acta Physiologiae Plantarum* **36**, 179–190 (2014).
 55. Ma, D. *et al.* Evaluation of yield, processing quality, and nutritional quality in different-colored wheat grains under nitrogen and phosphorus fertilizer application. *Crop Science* **58**, 402–415 (2018).
 56. Campos, C. N. S. *et al.* Macronutrient deficiency in cucumber plants: impacts in nutrition, growth and symptoms. *Journal of Plant Nutrition* **0**, 1–18 (2021).
 57. Osório, C. R. W. de S. *et al.* Macronutrient deficiency in snap bean considering physiological, nutritional, and growth aspects. *PLoS ONE* **15**, 1–15 (2020).
 58. Sales, A. C. *et al.* Silicon mitigates nutritional stress in quinoa (*Chenopodium quinoa* Willd.). *Scientific Reports* **11**, 1–16 (2021).
 59. Cao, B. L., Ma, Q., Zhao, Q., Wang, L. & Xu, K. Effects of silicon on absorbed light allocation, antioxidant enzymes and ultrastructure of chloroplasts in tomato leaves under simulated drought stress. *Scientia Horticulturae* **194**, 53–62 (2015).
 60. Berger, S., Sinha, A. K. & Roitsch, T. Plant physiology meets phytopathology: Plant primary metabolism and plant-pathogen interactions. *Journal of Experimental Botany* vol. 58 4019–4026 (2007).
 61. Mali, M. & Aery, N. C. Influence of silicon on growth, relative water contents and uptake of silicon, calcium and potassium in wheat grown in nutrient solution. *Journal of Plant Nutrition* **31**, 1867–1876 (2008).
 62. Hepler, P. K. & Winship, L. J. Calcium at the cell wall-cytoplasm interface. *Journal of Integrative Plant Biology* **52**, 147–160 (2010).
 63. Yoshida, S. Chemical aspects of the role of silicon in physiology of the rice plant. *Bulletin of the National Institute of Agricultural Sciences Series B* **15**, 1–58 (1965)

Figures

Figure 1

Silicon (Si) accumulation in the shoot (a, c) and roots (b, d) of *Urochloa brizantha* cv. Marandu and *Megathyrsus maximum* cv. Massai under grown in complete solution (CS) and deficiency of nitrogen (-N), phosphorus (-P) and calcium (-Ca), combined with Si (+Si) and without Si (-Si). Different letters indicate a significant difference between treatments in the +Si and -Si condition. *= Indicates a significant difference between treatments with nutritional deficiencies in relation to treatments with CS, by the Tukey test ($p < 0.05$).

Figure 2

Electrolyte leakage index (a, c) and phenolic compounds (b, d) of *Urochloa brizantha* cv. Marandu and *Megathyrsus maximum* cv. Massai under grown in complete solution (CS) and deficiency of nitrogen (-N), phosphorus (-P) and calcium (-Ca), combined with Si (+Si) and without Si (-Si). Different letters indicate a significant difference between treatments in the +Si and -Si condition. *= Indicates a significant difference between treatments with nutritional deficiencies in relation to treatments with CS. For the electrolyte leakage index the treatments of CS (l.2) are compared with the treatments - N (l.2), - P (l.2), refer to values obtained by the evaluation of the second fully expanded sheet. The treatments of CS (l.1) are compared with the values of - Ca (l.1) and refer to the first leaf. The comparison of means was performed using the Tukey test ($p < 0.05$).

Figure 3

Green color index (a, d) and Fv/Fm (b, d) of *Urochloa brizantha* cv. Marandu and *Megathyrsus maximum* cv. Massai under grown in complete solution (CS) and deficiency of nitrogen (-N), phosphorus (-P) and calcium (-Ca), combined with Si (+Si) and without Si (-Si). Different letters indicate a significant difference between treatments in the +Si and -Si condition. *= Indicates a significant difference between treatments with nutritional deficiencies in relation to treatments with CS. The treatments of CS (l.2) are compared with the treatments - N (l.2), - P (l.2), refer to values obtained by the evaluation of the second fully expanded sheet. The treatments of CS (l.1) are compared with the values of -Ca (l.1) and refer to the first leaf. The comparison of means was performed using the Tukey test ($p < 0.05$).

Figure 4

Efficiency of use the nitrogen (N) (a, d), phosphorus (P) (b, e) and calcium (Ca) (c, f) of *Urochloa brizantha* cv. Marandu and *Megathyrsus maximum* cv. Massai under grown in complete solution (CS) and deficiency of nitrogen (-N), phosphorus (-P) and calcium (-Ca), combined with Si (+Si) and without Si (-Si). Different letters indicate a significant difference between treatments in the +Si and -Si condition. *=

Indicates a significant difference between treatments with nutritional deficiencies in relation to treatments with CS, by the Tukey test ($p<0.05$).

Figure 5

Plant Length (a, d), number of tillers (b, e) and shoot dry matter (c, f) of plants of *Urochloa brizantha* cv. Marandu and *Megathyrsus maximum* cv. Massai under grown in complete solution (CS) and deficiency of nitrogen (-N), phosphorus (-P) and calcium (-Ca), combined with Si (+Si) and without Si (-Si). Different

letters indicate a significant difference between treatments in the +Si and -Si condition. *= Indicates a significant difference between treatments with nutritional deficiencies in relation to treatments with CS, by the Tukey test ($p < 0.05$).