MINISTÉRIO DA EDUCAÇÃO
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PROGRAMA DE PÓS-GRADUAÇÃO EM AGRONOMIA

LUCÉLIA ROSA DE JESUS

SILICON REDUCES ALUMINUM ACCUMULATION AND MITIGATES TOXIC EFFECTS IN COWPEA PLANTS

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Dissertation submitted to Federal Rural University of Amazônia, as part of the requirements for obtaining the *Magister Scientiae degree in Agronomy.*
Advisor: Prof. Dr. Allan Klynger da Silva Lobato

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Dissertation presented to the Federal Rural University of Amazonia, as part of the requirements of the Master Course in Agrarian Sciences: area of concentration in Agronomy, to obtain the title of Master.
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SUMMARY

RESUMO

Alumínio (Al) é o terceiro metal mais abundante na superfície da Terra, e Al toxicidade promove vários efeitos negativos no metabolismo das plantas. O silício (Si) é o segundo mineral mais comum no solo e é considerado um elemento benéfico para as plantas, melhorando sua tolerância aos estresses bióticos e abióticos. O objetivo deste estudo é determinar se o Si pode reduzir a acumulação de Al e explicar a possível contribuição do Si para mitigar a toxicidade de Al. O experimento teve um desenho fatorial com dois níveis de alumínio (0 e 10 mM Al) e três níveis de silício (0, 1,25 e 2,50 mM Si). A utilização de Si em plantas expostas à toxicidade de Al contribuiu para reduções significativas nos teores de Al de todos os tecidos, correspondendo a reduções de 51%, 29% e 41% em raízes, caules e folhas, respectivamente, após tratamento com Si 2,50 mM Al + 10 mM comparado com o tratamento de controlo (Si 0 mM + Al 10 mM). A toxicidade de Al promoveu diminuições no rendimento quântico efetivo do fotossistema II (ΦPSII), na dissipação fotoquímica (qP) e na taxa de transporte de elétrons (ETR), enquanto que 2,50 mM de Si induziram aumentos de 54%, 185% e 29%, respectivamente. As plantas expostas a Al apresentaram valores mais baixos na taxa de fotossíntese líquida (P_N), no uso eficiente da água (que) e na eficiência na carboxilação instantânea (P_N/C_i), enquanto a aplicação de Si a uma concentração de 2,50 mM apresentou melhorias de 53%, 32% e 67%, respectivamente. A exposição ao Al aumentou as atividades da superóxido dismutase (SOD), catalase (CAT), ascorbato peroxidase (APX) e peroxidase (POX), enquanto que o tratamento com Si 2,50 mM + Al 10 mM produziu variações significativas de 72%, 97%, 48% e 32%, respectivamente, em comparação com 0 mM de Si + 10 mM de Al. Nossos resultados mostraram que Si reduziu o conteúdo de Al e os fatores de bioconcentração para Al em todos os tecidos. Si também melhorou a eficiência fotoquímica de PSII, troca gasosa, pigmentos e enzimas antioxidantes, contribuindo para uma redução na acumulação de compostos oxidativos. Esses benefícios corroboram os múltiplos papéis exercidos pelo Si no metabolismo e revelam que Si imobiliza o Al nas raízes e reduz o acúmulo deste metal em outros órgãos, atenuando os danos causados pelo Al em plantas de caupi. Em relação à dose-resposta, as plantas expostas a Si 1,25 mM sem Al apresentaram melhores resultados em termos de crescimento, enquanto que os efeitos tóxicos de plantas expostas a Al foram mitigados com Si 2,50 mM.

PALAVRAS-CHAVE: Elemento benéfico. Crescimento. Toxicidade do metal. Eficiência fotoquímica do PSII. *Vigna unguiculata*.

ABSTRACT

Aluminum (Al) is the third most abundant metal in the Earth's surface, and Al toxicity promotes several negative effects in plant metabolism. Silicon (Si) is the second most abundant mineral in soil and is considered a beneficial element for plants, improving their tolerance to biotic and abiotic stresses. The aim of this study is to determine if Si can reduce the accumulation of Al and explain the possible contribution of the Si to mitigate Al toxicity. The experiment had a factorial design with two levels of aluminium (0 and 10 mM Al) and three levels of silicon (0, 1.25 and 2.50 mM Si). The utilization of Si in plants exposed to Al toxicity contributed to significant reductions in the Al contents of all tissues, corresponding to reductions of 51%, 29% and 41% in roots, stems and leaves, respectively, upon treatment with 2.50 mM Si + 10 mM Al compared to the control treatment (0 mM Si + 10 mM Al). Al toxicity promoted decreases in effective quantum yield of PSII photochemistry (ΦPSII), photochemical quenching (qP) and electron transport rate (ETR), whereas 2.50 mM Si induced increases of 54%, 185% and 29%, respectively. Plants exposed to Al had lower values of net photosynthetic rate (P_N), water-use efficiency (WUE) and instantaneous carboxylation efficiency (P_N/C_i), whereas Si application at a concentration of 2.50 mM yielded improvements of 53%, 32% and 67%, respectively. Al exposure increased superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX) and peroxidase (POX) activities, whereas treatment with 2.50 mM Si + 10 mM Al produced significant variations of 72%, 97%, 48% and 32%, respectively, compared to 0 mM Si + 10 mM Al. Our results proved that Si reduced the Al contents and bioconcentration factors for Al in all tissues. Si also improved the photochemical efficiency of PSII, gas exchange, pigments and antioxidant enzymes, contributing to a reduction in the accumulation of oxidative compounds. These benefits corroborate the multiple roles exercised by Si in metabolism and reveal that Si immobilizes the Al in roots and reduce the accumulation of this metal in other organs, mitigating the damage caused by Al in cowpea plants. In relation to dose-response, plants exposed to 1.25 mM Si without Al presented better results in terms of growth, whereas the toxic effects of plants exposed to Al were mitigated with 2.50 mM Si.

KEYWORDS: Beneficial element. Growth. Metal toxicity. Photochemical efficiency of PSII. *Vigna unguiculata*.

1 CONTEXTUALIZATION

Cowpea [*Vigna unguiculata* (L.) Walp.] it is an important leguminous, the grains contain on average 23-25% of proteins and 50-67% of carbohydrates, making this a promising food ingredient besides being an important generator of employment and income (FREIRE FILHO et al., 2011; DEVI et al., 2015). This crop is widely cultivated in Asia, South America and Africa performing well in tropical and subtropical regions (VENDRAMINI et al., 2011; KAPRAVELOU et al., 2015) and for its nutritional value is grown mainly for the production of grains, dried or green for human consumption in natura in the form of preserved or dehydrated (MORAIS et al., 2013).

The main producers of cowpea are Nigeria, Niger and Brazil (FROTA et al., 2008). In Brazil, the cowpea represents on average 20% of all bean production (MORAIS et al., 2013), with a planted area of approximately 1,249 ha, with emphasis on the North and Northeast regions being the largest producers in Brazil. In the North region, the average productivity is 2,116 kg ha^{-1}, the Pará State is being the second largest producer in this region with a mean productivity of 746 kg ha^{-1} in 2016, contributing 35% to the total production in the North region (CONAB, 2016).

Although the cultivation of cowpea presents great economic potential for grain production, its productivity can be strongly affected by the biotic and abiotic factors, which negatively influenced its growth and development (FREITAS et al., 2014) among the abiotic factors, (OKE and EYITAYO 2010), metal stress (GILL and TUTEJA 2010) and amid others.

In tropical soils where there is high rainfall, some soluble nutrients such as calcium, magnesium, potassium and other elements are easily leached, also occurring the mineralization of organic matter by soil microorganisms, resulting in the release of nitrate and hydrogen, such events favor the reduction of soil pH. With low pH, hydrogen ions (H^+) act on minerals releasing aluminum ions (Al^{3+}), so acidic soils (pH<5.0) favors the appearance of Al^{3+} the most toxic form to plant growth, being one of the main limiting factors to the production of most species grown on acid soils (ECHART and CAVALLI-MOLINA, 2001; BRITEZ et al., 2002). Aluminum comes up as a metal that can generate several negative effects on physiological processes in various cultures.

Glycine max and *Vigna radiata*, when exposed to Al toxicity, the inhibition of root length observed by the reduction of the cell expansion in the zone of elongation was detected, besides promoting the rupture of the external epidermal and cortical cells closer to the root tip (BLAMEY et al., 2005).

Another negative effects promoted by Al action were reported as increases in oxidative stress in *Oryza sativa* seedlings (BHOOMIKA et al., 2013) in *Sorghum bicolor* cultivars were affected by chlorophyll fluorescence and photosynthetic rates (PEIXOTO et al., 2002), Al also promoted a decrease

in nutrient content (K, P, Ca and Mg), leading to mineral imbalance in *Theobroma cacao* genotypes (RIBEIRO et al., 2013), both effects make this a major limiting factor for growth and productivity in several crops including cowpea.

The researches have been advancing in technique that aim to reduce the negative effects promoted by biotic and abiotic stresses, where silicon has emerged as an alternative to relieve these stresses. The use of silicate sources has been used to ameliorate pathogens infection (GHAREEB et al., 2011), water deficit (CAO et al., 2015), as well as to use agronomic potential. Several studies have demonstrated the beneficial effect of Si on mitigating the negative effects promoted by metals including Al in *Arachis hypogaea* (SHEN et al., 2014), chromium (Cr) in *Hordeum vulgare* (ALI et al., 2013), cadmium (Cd) in *Solanum nigrum* (LIU et al., 2013), Zinc (Zn) in *Oryza sativa* (GU et al., 2012). Thus, there are strong indications that Si may act to alleviate the stress promoted by Al in cowpea, but studies are scarce in understanding the action of Si on plants exposed to Al toxicity.

The aim of this study is to determine if Si can reduce the accumulation of Al and explain the possible contribution of the Si to mitigate Al toxicity.

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2 LITERTURE REVIEW

2.1 General aspects of cowpea

Cowpea (*Vigna unguiculata*) belonging to the Fabaceae family is a legume of African origin that presents several economic, agronomic and environmental advantages (WAWIRE et al. 2011; GONÇALVES et al., 2016). Introduced in Brazil in the mid-sixteenth century by the Portuguese settlers in the State of Bahia being disseminated throughout the country (FREIRE FILHO 2011). It is popularly known as string bean or macassar bean being an excellent source of protein (23% -25% on average) featuring all essential amino acids, carbohydrates (62% on average), vitamins and minerals as well as not containing cholesterol and for its rich nutritional value is commonly produced for the production of dry and green grains being used in human food (THARANATHAM and MAHADEVAMMA 2003; FREIRE FILHO et al., 2011).

This culture is widely cultivated in Asia, South America and Africa (KAPRAVELOU et al., 2015) and has performed well in tropical and subtropical regions (VANDRAMINI et al., 2011). In Brazil, the production of cowpea is concentrated in the North and Northeast, being an important generator of employment and income (FREIRE FILHO et al., 2011). In the North and Northeast, cowpea is widely accepted, comprising 9.5% of the cultivated area (MAPA, 2016).

The cowpea can be differentiated both by the growth habit, highlighting the decumbent, semi-erect and erect genotypes (WANG et al., 2007) and the existing types of grains varying according to size and different forms, such as the bean (ANDRADE JÚNIOR et al., 2002) and butter beans, being this an important grain in cooking in the Northeast region (FREIRE FILHO et al., 2011).

2.2 Aluminium in soil and plant

Aluminium is one of the most abundant metal in the earth's crust, non-phytotoxic forms such as

aluminosilicates and precipitates can be found. However, in acidic soils, biologically inactive forms of Al become highly toxic after solubilization (soluble Al^{3+}) at pH (<5.0), which can cause great damage to the plant production even in micromolar concentrations, making this element a major constraint on agricultural production (CHEN et al. 2008; CAO et al., 2011).

Usually soil acidification can develop naturally through the leaching of the basic cations, occurring mainly in tropical and subtropical soils with high rainfall, soluble nutrients such as calcium, magnesium, potassium and other basic elements are leached, allowing the pH decrease from soil. Mineralization of organic matter by soil microorganisms results in the release and nitrate and hydrogen, causing the pH decrease (ECHART and CAVALLI-MOLINA 2001). At low pH, the hydrogen (H^+) acts on mineral releasing aluminum ions (Al^{3+}) therefore, the amount of Al^{3+} in the solution increases with the acidity of the soil (BOHNEN, 1995).

Generally soil acidification can develop naturally through the leaching of the basic cations but can also be accelerated by some agricultural practices such as acid rain, containing high levels of exchangeable Al. In soils with near pH the neutrality favors the form of aluminum hydroxide ($Al(OH)_3$). Many cations of the monomeric Al can bind to various organic and inorganic binders such as PO_4^{3-}, SO_4^{2-}, organic acids, proteins and lipids (DELHAIZE and RYAN 1995; HE et al., 2011).

Aluminum (Al^{3+}) absorbed by plants can generate several disturbances in metabolism among others. First symptom of aluminum toxicity in plants is the inhibition of root growth, which can be attributed to the rupture the cell division in the meristematic region and the cell expansion in the zone of elongation of the roots. Causing a decrease in the epidermal turgescence of the root tip and the regions of elongation, favoring the appearance of large amounts of small depressions in the elongated regions, destruction of the epidermal cells and external cortex cells in the region of the portal and elongation and formation of transversal cracks In the internal cortex of the roots (WAGATSUMA et al., 1987;WU et al., 2014), both effects resulting in a reduced and damaged root system impairing the absorption of water and mineral nutrients (KOCHIAN et al., 2004; SILVA et al., 2012). In cultivars of *Oryza sativa* there was a reduction in root length when exposed to the concentration of 80 μM of Al, suggesting changes in the constituents of the cell wall, promoting the stiffness of the DNA double helix reducing the cellular division besides the root being the site of synthesis of Hormones such as cytokinins (MERIGA et al., 2010).

The toxicity of Al causes several damages to the plant metabolism, among them: the malformation of the chloroplast structure, affects mitochondrial functioning due to the production of reactive oxygen species (ROS), causing ATP depletion and respiratory stress, favoring the perturbation to the photosynthetic apparatus (MOUSTAKAS et al., 1996; INOSTROZA - BLANCHETEAU et al., 2011).

The induction of ROS, causing membrane peroxidative damage (KOCHIAN et al., 2004). A potential target for aluminum is the plasma membrane, as it changes the physical properties of the membrane through the interaction with the ATPase and lipids in the membranes (IMADI et al., 2016).

Aluminum still affects negatively the gas exchanges, like increases in Ci, and this phenomenon can be attributed to Al action in promoting changes in the subunits the RUBISCO enzyme (ZHOU et al., 2009).

The absorption of Al may promote the mineral imbalance of macronutrients and micronutrients in some crops. Cations such as Ca and Mg compete for the same Al adsorption site, therefore, plants exposed to aluminum can express deficiency of these elements as occurred in Zea mays lineage sensitive to Al (GIONNAKOULA et al., 2008). Some micronutrients can also be reduced when plants are submitted to aluminum, in Oryza sativa leaves Mn and Zn contents were reduced due to the action of Al (SINGH et al., 2011).

Oxidative stress is also an effect caused by the action of aluminum, occurring when there is an imbalance between ROS production and its elimination by the antioxidant system (APEL and HIRT 2004). Several compounds are generated in the stress phase when subjected to aluminum as superoxide radical (O_2^-) and hydrogen peroxide (H_2O_2). This is formed after the action of the enzyme superoxide dismutase (SOD) (DONG et al., 2002; PEREIRA et al., 2011; BHOOMIKA et al., 2013).

Several of these harmful effects caused by aluminum contribute to the reduction of both root and shoot growth, strongly influences the production of biomass (BANHOS et al., 2016).

2.3 Silicon in soil and plant

Silicon (Si) is the second most abundant element in the soil, after oxygen, and is most commonly found in the form of Silicic Acid (H_4SiO_4). Most are in the non-dissociated form, which is readily available and absorbed by plants in the form of H_4SiO_4 (CHEN et al., 2000, MA et al., 2001, ABRO et al., 2009).

Silicon, when absorbed by plants, can accumulate below the epidermis, forming a layer called silica cell, deposited as hydrated amorphous silica (SiO_2nH_2O) (DAGMAR et al., 2003). However, the concentration of Si in plant tissues varies according to the absorption and transport, differentiating according to the species, and translocated from the root to the area by way of xylem (MA and YAMAJI, 2006). However, most plants absorb Si by passive diffusion, so that Si reaches the xylem and reaches the aerial part following the flow of transpiration. Absorption of Si may occur actively or passive diffusion (MA et al., 2007; MA and YAMAJI 2008). The build-up may range from 0.1% to 10% of its dry weight (MA et al., 2006).

The element is considered to be beneficial for plant growth, helping them to overcome biotic and abiotic stresses such as reduction of damage promoted by pathogens (GHAREEB et al., 2011), saline stress relief (ROMERO-ARANDA et al., 2006), Stress caused by water deficit (CAO et al., 2015), besides increasing the tolerance of the plants to the metal toxicity, including the Al in plants of *Zea mays* (KIDD et al., 2001), manganese in Zea mays (DONCHEVA et al., 2009), zinc in *Oryza sativa* (GU et al., 2012), chromium in *Hordeum vulgare* (ALI et al., 2013), cadmium in *Zea mays* (DRESLER et al., 2015) as well as iron deficiency relief in *Cucumis sativus* (PAVLOVIC et al., 2013).

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ARTICLE

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ORIGINAL ARTICLE

Silicon reduces aluminum accumulation and mitigates toxic effects in cowpea plants

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Abstract Aluminum (Al) is the third most abundant metal in the Earth's surface, and Al toxicity promotes several negative effects in plant metabolism. Silicon (Si) is the second most common mineral in soil and is considered a beneficial element for plants, improving their tolerance to biotic and abiotic stresses. The aim of this study is to determine whether Si can reduce the accumulation of Al, explain the possible contribution of Si in mitigating Al toxicity, and indicate the better Si dose–response for cowpea plants. The experiment had a factorial design with two levels of aluminum (0 and 10 mM Al) and three levels of silicon (0, 1.25 and 2.50 mM Si). The utilization of Si in plants exposed to Al toxicity contributed to significant reductions in the Al contents of all tissues, corresponding to reductions of 51, 29 and 41% in roots, stems and leaves, respectively, upon treatment with 2.50 mM Si + 10 mM Al compared to the control treatment (0 mM Si + 10 mM Al). Al toxicity promoted decreases in Φ_{PSII}, q_P and ETR, whereas 2.50 mM Si induced increases of 54, 185 and 29%, respectively. Plants exposed to Al had lower values of P_N, WUE and P_N/C_i, whereas Si application at a concentration of 2.50 mM yielded improvements of 53, 32 and 67%, respectively. Al exposure increased SOD, CAT, APX and POX activities, whereas treatment with 2.50 mM Si + 10 mM Al produced significant variations of 72, 97, 48 and 32%, respectively, compared to 0 mM Si + 10 mM Al. Our results proved that Si reduced the Al contents in all tissues. Si also improved the photochemical efficiency of PSII, gas exchange, pigments and antioxidant enzymes, contributing to a reduction in the accumulation of oxidative compounds. These benefits corroborate the multiple roles exercised by Si in metabolism and reveal that Si immobilizes the Al in roots and reduce the accumulation of this metal in other organs, mitigating the damage caused by Al in cowpea plants. In relation to dose–response, plants exposed to 1.25 mM Si without Al presented better results in terms of growth, whereas the toxic effects of plants exposed to Al were mitigated with 2.50 mM Si.

Keywords Beneficial element · Growth · Metal toxicity · Photochemical efficiency of PSII · *Vigna unguiculata*

Abbreviations

Φ_{PSII}	Effective quantum yield of PSII photochemistry
Al	Aluminium
AsA	Ascorbate
Ca	Calcium
CAR	Carotenoids
Chl*a*	Chlorophyll *a*
Chl*b*	Chlorophyll *b*
C_i	Intercellular CO_2 concentration
CO_2	Carbon dioxide
E	Transpiration rate
EL	Electrolyte leakage
ETR	Electron transport rate

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ETR/P_N	Ratio between the apparent electron transport rate and net photosynthetic rate
EXC	Relative energy excess at the PSII level
Fe	Iron
F_m	Maximal fluorescence yield of the dark-adapted state
F_0	Minimal fluorescence yield of the dark-adapted state
F_v	Variable fluorescence
F_v/F_m	Maximal quantum yield of PSII photochemistry
g_s	Stomatal conductance
H_2O_2	Hydrogen peroxide
K	Potassium
MDA	Malondialdehyde
Mg	Magnesium
Mn	Manganese
NPQ	Nonphotochemical quenching
O_2^-	Superoxide
P_N	Net photosynthetic rate
P_N/C_i	Instantaneous carboxylation efficiency
PSII	Photosystem II
q_P	Photochemical quenching
ROS	Reactive oxygen species
RUBISCO	ribulose-1,5-bisphosphate carboxylase/ oxygenase
Si	Silicon
Total Chl	Total chrolophyll
WUE	Water-use efficiency
Zn	Zinc

Introduction

The cowpea (*Vigna unguiculata* L.) is a legume widely cultivated in Asia, Africa and the Americas. The focus of this culture is grains, which are used in human nutrition and have high carbohydrate and protein contents (Demiate et al. 2016). Cultivars developed by breeding programmes often exhibit decreased production in acidic soils or without correction due to the increase in the availability of aluminum (Al) (Panda et al. 2009).

Al is the third most abundant metal in the Earth's surface. It is present in approximately 40% of the areas with agricultural potential (Chen et al. 2008). In acidic soils (pH<5.0), this element is soluble and available to plants in the form of Al^{3+}, which is toxic to various crops (Yang et al. 2013).

Al toxicity promotes several negative effects, such as the inhibition of root growth, a reduction in shoot length, nutritional imbalance, an increase in oxidative stress, limitations in gas exchange and a reduction in the synthesis of photosynthetic pigments, revealing that this element acts as a limiting factor in plant metabolism (He et al. 2011; Barros Júnior et al. 2016).

Silicon (Si) is a beneficial element for plants and is the second most common mineral in soil (Gu et al. 2012), occurring in the form of silica or silicate, which can be combined with various metals (Ma et al. 2011). Si can be absorbed by the roots in the form of silicic acid [$Si(OH)_4$], being transported via xylem to the shoot and the contents of this element in the shoot of the plant vary between 0.1 and 10% dry matter (Liang et al. 2007).

Si mitigates biotic and abiotic stresses, such as infection by a pathogen, saline stress and drought stress, increasing the tolerance of plants to metal toxicity, including Al in *Zea mays* plants (Kidd et al. 2001), manganese in *Zea mays* (Doncheva et al. 2009), zinc in *Oryza sativa* (Gu et al. 2012), chromium in *Hordeum vulgare* (Ali et al. 2013) and cadmium in *Zea mays* (Dresler et al. 2015), as well as alleviating the iron deficiency in *Cucumis sativus* (Pavlovic et al. 2013).

In the literature, there are few studies on the interaction between the Si x Al. The available studies describe the increase in the absorption of nutrients in *Oryza sativa* (Singh et al. 2011), reduction of oxidative damage in *Arachis hypogaea* (Shen et al. 2014) and improvements in the gas exchange of *Eucalyptus platyphylla* (Lima et al. 2016), suggesting that Si can mitigate Al toxicity. Our hypothesis was based on the negative interference promoted by Al and the benefits induced by Si in plant metabolism. The aim of this study is to determine if Si can reduce the accumulation of Al, explain the possible contribution of the Si to mitigate Al toxicity, and indicate the better Si dose–response for cowpea plants.

Materials and methods

Location and growth conditions

The experiment was performed at the Campus of Paragominas of the Universidade Federal Rural da Amazônia, Paragominas, Brazil (2°55^{0}S, 47°34^{0}W). The study was conducted in a greenhouse with the temperature and humidity controlled. The minimum, maximum, and median temperatures were 23, 32 and 26.5 °C, respectively. The relative humidity during the experimental period varied between 60 and 80%.

Plants, containers and acclimation

Seeds of *Vigna unguiculata* L. cv. BR3-Tracuateua were germinated and grown in 1.2-L pots (0.15 m in height and 0.10 m in diameter) filled with a mixed substrate of sand and vermiculite at a ratio of 3:1. The plants were cultivated under semi-hydroponic conditions, and the pots had one hole in the bottom covered with mesh to maintain the

substrate and aerate the roots. Solution absorption was by capillary action, with these pots placed into other containers (0.15 m in height and 0.15 m in diameter) containing 500 ml of distilled water for five days. A modified nutritive solution was used for nutrients, and the ionic force began at 50% and was modified to 100% after one day. After one day, the nutritive solution remained at total ionic force.

Experimental design

The experiment was a factorial design with the factors completely randomized, with two levels of aluminum (0 and 10 mM Al, being described as -Al and +Al, respectively) and three levels of silicon (0, 1.25 and 2.50 mM Si). With five replicates for each of the six treatments, a total of 30 experimental units were used in the experiment, with one plant in each unit.

Plant conduction and treatments with Si and Al

Six-day-old plants received the following macro- and micronutrients from the nutritive solution: 8.75 mM KNO_3, 7.5 mM $Ca(NO_3)_2 \cdot 4H_2O$, 3.25 mM $NH_4H_2PO_4$, 1.5 mM $MgSO_4 \cdot 7\,H_2O$, 62.50 µM KCl, 31.25 µM H_3BO_3, 2.50 µM $MnSO_4 \cdot H_2O$, 2.50 µM $ZnSO_4 \cdot 7H_2O$, 0.63 µM $CuSO_4 \cdot 5H_2O$, 0.63 µM $NaMoO_4 \cdot 5H_2O$, and 250.0 µM $NaEDTAFe \cdot 3H_2O$. One plant per pot was used during plant conduction. For the Si treatment, $Na_2SiO_3 \cdot 9H_2O$ was pre-solubilized with H_2SO_4, being used at concentrations of 0, 1.25 and 2.50 mM Si applied over 26 days (days 6–32 after the start of the experiment). To simulate Al exposure, $AlCl_3$ was used at concentrations of 0 and 10 mM Al, which was applied over 16 days (days 16–32 after the start of the experiment). During the study, the solutions were changed at 07:00 h in 3-day intervals, with the pH adjusted daily to 4.5 using HCl or NaOH. All reagents used in this study were obtained from Sigma-Aldrich™. On day 32 of the experiment, physiological and morphological parame- ters were measured for all plants, and leaf tissues were harvested for nutritional and biochemical analyses (Fig. S1 and S2).

Measurement of chlorophyll fluorescence

The minimal fluorescence yield of the dark-adapted state (F_0), the maximal fluorescence yield of the dark-adapted state (F_m), the variable fluorescence (F_v), the maximal quantum yield of PSII photochemistry(F_v/F_m), the effective quantum yield of PSII photochemistry (Φ_{PSII}), the photochemical quenching coefficient (q_P), the nonphotochemical quenching (NPQ), the electron transport rate (ETR), the relative energy excess at the PSII level (EXC) and the ratio between the electron transport rate and the net photosynthetic rate (ETR/P_N) were determined using a modulated chlorophyll fluorometer (model OS5p; Opti-Sciences). Chlorophyll fluorescence was measured in fully expanded leaves under light. Preliminary tests determined the location of the leaf, the part of the leaf and the time required to obtain the greatest F_v/F_m ratio; therefore, the acropetal third of leaves that were in the middle third of the plant and adapted to the dark for 30 min was used in the evaluation. The intensity and duration of the saturation light pulse were 7500 µ mol m^{-2} s^{-1} and 0.7 s, respectively.

Evaluation of gas exchange

The net photosynthetic rate (P_N), transpiration rate (E), stomatal conductance (g_s), and intercellular CO_2 concentration (C_i) were evaluated using an infrared gas analyser (model LCPro$^+$; ADC BioScientific). These parameters were measured at the adaxial surface of fully expanded leaves that were collected from the middle region of the plant. The water-use efficiency (WUE) was estimated according to Ma et al. (2004), and the instantaneous carboxylation efficiency (P_N/C_i). Gas exchange was evaluated in all plants under constant conditions of CO_2 concentration, photosynthetically active radiation, air-flow rate and temperature in a chamber at 360 µmol mol^{-1} CO_2, 800 µmol photons m^{-2} s^{-1}, 300 µmol s^{-1} and 28 °C, respectively, between 10:00 and 12:00 h.

Extraction of antioxidant enzymes, superoxide and soluble proteins

Antioxidant enzymes (SOD, CAT, APX and POX), superoxide and soluble proteins were extracted from leaf tissue as per the method of Badawi et al. (2004). The extraction mixture was prepared by homogenizing 500 mg of fresh plant material in 5 ml of extraction buffer, consisting of 50 mM phosphate buffer (pH 7.6), 1.0 mM ascorbate and 1.0 mM EDTA. Samples were centrifuged at 14,0009g for 4 min at 3 °C, and the supernatant was collected. The total soluble proteins were quantified using the method described by Bradford (1976). The absorbance was measured at 595 nm using bovine albumin as the standard.

Superoxide dismutase assay

For the SOD assay (EC 1.15.1.1), 2.8 ml of reaction mixture containing 50 mM phosphate buffer (pH 7.6), 0.1 mM EDTA, 13 mM methionine (pH 7.6), 75 µM NBT, and 4 µM riboflavin was mixed with 0.2 ml of supernatant. The absorbance was then measured at 560 nm (Giannopolitis

and Ries 1977). One SOD unit was defined as the amount of enzyme required to inhibit 50% of the NBT photoreduction. The SOD activity was expressed in unit mg^{-1} protein.

Catalase assay

For the CAT assay (EC 1.11.1.6), 0.2 ml of supernatant and 1.8 ml of reaction mixture containing 50 mM phosphate buffer (pH 7.0) and 12.5 mM hydrogen peroxide were mixed, and the absorbance was measured at 240 nm (Havir and McHale 1987). The CAT activity was expressed in µmol H_2O_2 mg^{-1} protein min^{-1}.

Ascorbate peroxidase assay

For the APX assay (EC 1.11.1.11), 1.8 ml of reaction mixture containing 50 mM phosphate buffer (pH 7.0), 0.5 mM ascorbate, 0.1 mM EDTA, and 1.0 mM hydrogen peroxide were mixed with 0.2 ml of supernatant, and the absorbance was measured at 290 nm (Nakano and Asada 1981). The APX activity was expressed in µmol AsA mg^{-1} protein min^{-1}.

Peroxidase assay

For the POX assay (EC 1.11.1.7), 1.78 ml of reaction mixture containing a 50 mM phosphate buffer (pH 7.0) and 0.05% guaiacol was mixed with 0.2 ml of supernatant, followed by the addition of 20 µl of 10 mM hydrogen peroxide. The absorbance was then measured at 470 nm (Cakmak and Marschner 1992). The POX activity was expressed in µmol tetraguaiacol mg^{-1} protein min^{-1}.

Determination of superoxide concentration

To determine O_2^-, 1 ml of extract was incubated with 30 mM phosphate buffer [pH 7.6] and 0.51 mM hydroxylamine hydrochloride for 20 min at 25 °C. Then, 17 mM sulphanilamide and 7 mM α-naphthylamine were added to the incubation mixture for 20 min at 25 °C. After the reaction, ethyl ether was added in the identical volume and centrifuged at 3000 × g for 5 min. The absorbance was measured at 530 nm (Elstner and Heupel 1976).

Extraction of nonenzymatic compounds

Nonenzymatic compounds (H_2O_2 and MDA) were extracted as described by Wu et al. (2006). Briefly, a mixture for extraction of H_2O_2 and MDA was prepared by homogenizing 500 mg of fresh leaf materials in 5 ml of 5% (w/v) trichloroacetic acid. Then, the samples were centrifuged at 15,000 × g for 15 min at 3 °C to collect the supernatant.

Determination of hydrogen peroxide concentration

To measure H_2O_2, 200 µl of supernatant and 1800 µl of reaction mixture (2.5 mM potassium phosphate buffer [pH 7.0] and 500 mM potassium iodide) were mixed, and the absorbance was measured at 390 nm (Velikova et al. 2000).

Quantification of malondialdehyde concentration

MDA was determined by mixing 500 µl of supernatant with 1000 µl of the reaction mixture, which contained 0.5% (w/v) thiobarbituric acid in 20% trichloroacetic acid. The mixture was incubated in boiling water at 95 °C for 20 min, with the reaction terminated by placing the reaction container in an ice bath. The samples were centrifuged at 10,000 × g for 10 min, and the absorbance was measured at 532 nm. The nonspecific absorption at 600 nm was subtracted from the absorbance data. The MDA–TBA complex (red pigment) amount was calculated based on the method of Cakmak and Horst (1991), with minor modifications and using an extinction coefficient of 155 mM^{-1} cm^{-1}.

Determination of electrolyte leakage

Electrolyte leakage was measured according to the method of Gong et al. (1998) with minor modifications. Fresh tissue (200 mg) was cut into pieces 1 cm in length and placed in containers with 8 ml of distilled deionised water. The containers were incubated in a water bath at 40 °C for 30 min, and the initial electrical conductivity of the medium (EC_1) was measured. Then, the samples were boiled at 95 °C for 20 min to release the electrolytes. After cooling, the final electrical conductivity (EC_2) was measured (Gong et al. 1998). The percentage of electrolyte leakage was calculated using the formula EL (%) = (EC_1/EC_2) × 100.

Determination of photosynthetic pigments

The chlorophyll and carotenoid determinations were performed with 40 mg of leaf tissue. The samples were homogenized in the dark with 8 ml of 90% methanol (Nuclear). The homogenate was centrifuged at 6000 × g for 10 min at 5 °C. The supernatant was removed, and chlorophyll a (Chl *a*) and *b* (Chl *b*), carotenoid (Car) and total chlorophyll (total Chl) contents were quantified using a spectrophotometer (model UV-M51; Bel Photonics), according to the methodology of Lichtenthaler and Buschmann (2001).

Measurements of morphological parameters

The growth of roots, stems and leaves was measured based on constant dry weights (g) after drying in a forced-air ventilation oven at 65 °C.

Extraction and Si determination

Samples containing 100 mg of dry matter were placed in a muffle furnace and kept for 3 h at 500 °C. The material was removed and mixed in 10 ml of 1% NaOH. For Si determination, 200 µl of supernatant and 1720 µl of reaction mixture (0.078 N HCl, 3.45 mM $NH_4Mo_7O_{24}$, 54 mM tartaric acid) were mixed with an 80 µl of reducing agent. The reducing agent was prepared with 40 mM Na_2SO_3, 10.5 mM 1-amino-2-naphthol-4-sulfonic acid, and 1.45 mM $NaHSO_3$. The absorbance was measured at 600 nm (Ma et al. 2004).

Determination of Al and nutrients

Samples with 100 mg of milled samples were weighed in 50-ml conical tubes (Falcon^R, Corning, Mexico) and predigested (48 h) with 2 ml of sub boiled HNO_3 (DST 1000, Savillex, USA). After, 8 ml of a solution containing 4 ml of H_2O_2 (30% v/v, Synth, Brasil) and 4 ml of ultra-pure water (Milli-Q System, Millipore, USA) were added, and the mixture was transferred to a Teflon digestion vessel, closed and heated in a block digester (EasyDigest®, Analab, France) according to the following program: (a) 100 °C for 30 min; (b) 150 °C for 30 min; (c) 130 °C for 10 min; (d) 100 °C for 30 min and; and (e) left to cool. The volume was made to 50 ml with ultra-pure water, and iridium was used as an internal standard at 10 lg l^{-1}. The determination of Al, K, Ca, Mg, Mn, Fe and Zn was carried out using an inductively coupled plasma mass spectrometer (ICP-MS 7900, Agilent, USA). Certified reference materials (NIST 1570a and NIST 1577c) were run in each batch for quality control purposes. All found values were in agreement with certified values.

Data analysis

The data were subjected to an analysis of variance, and significant differences between the means were determined using the Scott–Knott test at a probability level of 5%. Standard deviations were calculated for each treatment. The statistical analyses were performed with Assistat software.

Results

Al and Si contents in plants tissues

Si application in *V. unguiculata* plants exposed to Al toxicity contributed to significant reductions in Al content in the tissues (Table 1). These reductions correspond to 51, 29 and 41% in roots, stems and leaves, respectively, for the treatment with 2.50 mM Si + 10 mM Al compared to the control treatment (0 mM Si + 10 mM Al). In relation to Si contents, plants treated with Si exhibited increases ($P < 0.05$) in Si contents for all tissues evaluated (root, stem and leaf) in both conditions (with or without Al), with the contents being root > leaf > stem (Table 2).

Si contributed to increases in element contents in tissues

Al promoted significant reductions in the contents of macronutrients (K, Ca and Mg) and micronutrients (Mn, Fe and Zn) (Table 3). However, treatment with 2.50 mM Si + 10 mM Al induced increases in K, Ca and Mg contents of 74, 24 and 21% (root); 24, 38 and 43% (stem); and 155, 39 and 55% (leaf), respectively, compared to 0 mM Si + 10 mM Al. Additionally, plants exposed to a treatment of 2.50 mM Si + 10 mM Al showed increases in Mn, Fe and Zn of 45, 79 and 24% in roots, 27, 12 and 129% in stems and 34, 27 and 31% in leaves, respectively, compared to the control treatment exposed to Al toxicity.

Si mitigated the toxic effects of the Al on chlorophyll fluorescence

Al toxicity induced increases in F_0 values, but Si induced significant reductions of 28% compared to 0 mM Si + 10 mM Al (Fig. 1a). To F_m and F_v/F_m, the use of Al caused declines (Fig. 1b, c), but a Si concentration under 2.50 mM induced increases of 19 and 24%, respectively, compared to the control with 10 mM Al. The application of Al promoted significant reductions in U_{PSII}, q_P and ETR, whereas the concentration of 2.50 mM Si induced increases of 54, 185 and 29%, respectively, compared to the treatment 0 mM Si + 10 mM Al (Table 4). In plants without Al, the highest values of these variables were found for the concentration of 1.25 mM Si. NPQ, EXC and ETR/P_N showed increases when Al was applied, whereas 2.50 mM Si significantly reduced the values of these variables by 55, 12 and 15%, respectively, in comparison with 0 mM Si + 10 mM Al (Table 4). In addition, the lowest values of NPQ, EXC and ETR/P_N were reported for treatment with 1.25 mM Si + 0 mM Al.

Table 1 Al contents in *V. unguiculata* plants treated with Si and exposed to Al toxicity

Al (mM)	Si (mM)	Al content (μg g^{-1})		
		Root	Stem	Leaf
0	0	1.7 ± 0.2Ab	0.26 ± 0.07Ab	0.15 ± 0.06Ab
0	1.25	1.6 ± 0.1Ab	0.15 ± 0.05Ab	0.09 ± 0.04Ab
0	2.50	1.5 ± 0.2Ab	0.16 ± 0.02Ab	0.09 ± 0.05Ab
10	0	23,567.2 ± 2201.3Aa	466.17 ± 42.6Aa	430.77 ± 34.6Aa
10	1.25	17,100.4 ± 883.3Ba	354.06 ± 28.9Ba	341.76 ± 22.2Ba
10	2.50	11,608.5 ± 842.8Ca	330.67 ± 32.4Ba	254.33 ± 25.0Ca

Columns with different uppercase letters between Si levels (0, 1.25 and 2.50 mM Si under equal Al concentration) and lowercase letters between Al levels (0 and 10 mM Al under equal Si concentration) indicate significant differences from the Scott-Knott test ($P < 0.05$). Values described corresponding to means from five repetitions and standard deviations

Table 2 Si contents in *V. unguiculata* plants treated with Si and exposed to Al toxicity

Al (mM)	Si (mM)	Si content (μg g^{-1})		
		Root	Stem	Leaf
0	0	76 ± 27Ba	36 ± 15Ba	42 ± 14Ba
0	1.25	9890 ± 431Aa	7253 ± 391Aa	7854 ± 381Aa
0	2.50	10,293 ± 722Aa	7359 ± 532Aa	8042 ± 322Aa
10	0	40 ± 21Ba	18 ± 14Ca	20 ± 12Ca
10	1.25	6945 ± 501Ab	5385 ± 441Bb	5915 ± 261Bb
10	2.50	7074 ± 261Ab	6300 ± 211Ab	6840 ± 312Ab

Columns with different uppercase letters between Si levels (0, 1.25 and 2.50 mM Si under equal Al concentration) and lowercase letters between Al levels (0 and 10 mM Al under equal Si concentration) indicate significant differences from the Scott-Knott test ($P < 0.05$). Values described corresponding to means from five repetitions and standard deviations

Improvement induced by Si on gas exchange

Plants submitted to Al suffered reductions ($P < 0.05$) in P_N, WUE and P_N/C_i, but the application of Si at a concentration of 2.50 mM resulted in increases of 53, 32 and 67%, respectively, compared to the treatment 0 mM Si + 10 Al mM (Table 5). In P_N and P_N/C_i, the concentration of 1.25 mM Si in the absence of Al showed the highest values, with increases of 43 and 47%, respectively, compared to the treatment 0 mM Si + 0 mM Al. For E and g_s significant effects were observed between treatments without Al exposed to different concentrations of Si; in both variables, the highest values were observed upon exposure to 1.25 mM Si, with increments of 28 and 36%, respectively, in relation to 0 mM Si without Al (Table 5). Al caused an increase in C_i values, but the use of Si at a concentration of 2.50 mM caused a decrease in the values of this variable, with a decrease of 10%, in comparison with the treatment 0 mM Si + 10 mM Al (Table 5).

Si increases in antioxidant enzyme activities in plants under Al toxicity

The Al treatment causes increases in the SOD and CAT activities of cowpea plants, and treatment with 2.50 mM Si + 10 mM Al produced significant variations of 72 and 97%, respectively, compared to 0 mM Si + 10 mM Al (Fig. 2a, b). Regarding APX and POX, the stress caused by Al markedly increased the activity of these enzymes, and increases in Si concentrations also induced increases in APX and POX activities. Treatment with 2.50 mM Si + 10 mM Al presented increases of 48 and 32%, respectively, compared to the control with 10 mM Al (Fig. 2c, d).

Minor oxidative stress and cell damage promoted by the Si in plants exposed to Al

O_2^- and H_2O_2 showed strong increases in plants subjected to Al stress; however, the addition of 2.50 mM Si induced significant reductions of 22 and 16%, respectively,

Table 3 Nutrient contents in *V. unguiculata* plants treated with Si and exposed to Al toxicity

Al (mM)	Si (mM)	K (mg g DM^{-1})	Ca (mg g DM^{-1})	Mg (mg g DM^{-1})	Mn (mg g DM^{-1})	Fe (mg g DM^{-1})	Zn (mg g DM^{-1}
Contents in root							
0	0	23.60 ± 0.7Ca	0.42 ± 0.04Ba	43.10 ± 1.1Aa	340.37 ± 17.0Ba	340.43 ± 8.8Aa	64.18 ± 3.5Aa
0	1.25	25.41 ± 0.8Ba	0.46 ± 0.04Ba	44.00 ± 2.3Aa	344.07 ± 11.1Ba	341.80 ± 28.6Aa	64.53 ± 0.7Aa
0	2.50	30.52 ± 1.2Aa	0.61 ± 0.03Aa	44.11 ± 2.9Aa	375.93 ± 15.5Aa	342.70 ± 9.9Aa	64.64 ± 0.6Aa
10	0	15.58 ± 1.0Cb	0.42 ± 0.03Ba	21.88 ± 0.9Bb	129.33 ± 9.3Cb	119.60 ± 6.5Bb	24.64 ± 1.3Bb
10	1.25	23.48 ± 0.8Bb	0.38 ± 0.02Bb	23.96 ± 1.4Bb	158.94 ± 12.8Bb	214.13 ± 4.9Ab	27.35 ± 1.5Bb
10	2.50	27.10 ± 0.9Ab	0.52 ± 0.04Ab	26.40 ± 0.7Ab	187.40 ± 8.0Ab	214.20 ± 4.7Ab	30.60 ± 1.1Ab
Contents in stem							
0	0	37.92 ± 0.3Ba	0.44 ± 0.02Ba	2.85 ± 0.19Ba	25.81 ± 1.4Ca	45.16 ± 1.6Aa	12.90 ± 0.3Aa
0	1.25	42.30 ± 0.6Aa	0.52 ± 0.04Aa	3.36 ± 0.13Aa	30.43 ± 2.0Ba	47.00 ± 1.8Aa	13.04 ± 0.4Aa
0	2.50	42.06 ± 0.8Aa	0.55 ± 0.03Aa	3.48 ± 0.11Aa	36.93 ± 1.2Aa	47.07 ± 1.9Aa	13.14 ± 0.6Aa
10	0	28.91 ± 0.4Cb	0.37 ± 0.02Bb	1.91 ± 0.09Cb	21.08 ± 0.9Cb	23.90 ± 0.8Bb	4.05 ± 0.1Cb
10	1.25	30.14 ± 0.6Bb	0.48 ± 0.02Aa	2.38 ± 0.12Bb	23.91 ± 1.1Bb	24.58 ± 0.7Bb	7.96 ± 0.1Bb
10	2.50	35.75 ± 0.8Ab	0.51 ± 0.05Aa	2.73 ± 0.14Ab	26.77 ± 1.3Ab	26.68 ± 1.0Ab	9.29 ± 0.4Ab
Contents in leaf							
0	0	16.16 ± 0.9Ca	1.34 ± 0.04Ba	3.60 ± 0.10Aa	104.59 ± 2.2Aa	116.20 ± 5.2Aa	17.16 ± 0.5Aa
0	1.25	18.18 ± 0.8Ba	1.49 ± 0.07Aa	3.51 ± 0.06Aa	106.21 ± 2.0Aa	119.11 ± 4.7Aa	17.50 ± 1.0Aa
0	2.50	22.97 ± 0.9Aa	1.56 ± 0.11Aa	3.50 ± 0.07Aa	108.30 ± 3.6Aa	121.76 ± 5.9Aa	17.62 ± 0.1Aa
10	0	6.18 ± 0.5Cb	1.08 ± 0.03Cb	2.14 ± 0.07Cb	73.41 ± 1.9Cb	74.97 ± 4.3Bb	10.37 ± 0.2Cb
10	1.25	10.97 ± 0.1Bb	1.35 ± 0.05Bb	2.50 ± 0.11Bb	87.67 ± 3.6Bb	93.70 ± 4.5Ab	11.89 ± 0.5Bb
10	2.50	15.77 ± 0.1Ab	1.50 ± 0.06Aa	3.32 ± 0.21Aa	98.41 ± 1.9Ab	95.16 ± 2.2Ab	13.61 ± 0.5Ab

Columns with different uppercase letters between Si levels (0, 1.25 and 2.50 mM Si under equal Al concentration) and lowercase letters between Al levels (0 and 10 mM Al under equal Si concentration) indicate significant differences from the Scott–Knott test ($P < 0.05$). Values described corresponding to means from five repetitions and standard deviations

K potassium, Ca calcium, Mg magnesium, Mn manganese, Fe iron, Zn zinc

compared to treatment with 0 mM Si + 10 M Al (Fig. 3a, b). Al-induced increases in EL and MDA, but pretreatment with 2.50 mM Si significantly reduced the interference of Al stress in 37 and 15%, respectively, in comparison with 0 mM Si + 10 mM Al (Fig. 3c, d).

Si exogenous alleviated the Al interference on photosynthetic pigments

The Al-induced reductions in Chl *a*, Chl *b* and total Chl levels, as well as the application of 2.50 mM Si in the plants exposed to Al, caused significant increases of 34, 24 and 32%, respectively, compared to the treatment 0 mM Si + 10 Al mM (Fig. 4a–c). In addition, plants grown in the absence of Al had the highest values for Chl *a*, Chl *b*, total Chl and Car for treatment with 1.25 mM Si. In CAR, a significant increase of 20% was observed upon treatment with 1.25 mM Si + 0 mM Al compared to the control without Al. For plants treated with Al, applying 2.50 mM Si resulted in an increase of 13% in the CAR levels compared to 0 mM Si + 10 mM Al (Fig. 4d).

Benefits linked to Si action on growth

Treatments under the action of 1.25 mM Si + 0 mM Al had increases ($P < 0.05$) of 10, 19 and 32% in LDM, SDM and the TDM, respectively, compared to a treatment of 0 mM Si + 0 mM Al. In plants subjected to stress by Al, increases of 15, 24 and 22% were observed at a concentration of 2.50 mM Si compared to 0 mM Si (Table 6). For RDM, treatment 1.25 mM Si without Al caused an increase of 77%, compared to the control of 0 mM Si. The addition of Al promoted a reduction in this variable; however, the application of 2.50 mM Si revealed an increase of 30%, compared with treatment with 0 mM Si with Al (Table 6).

Discussion

The Al contents were reduced in all tissues after Si application, suggesting that Si interfered with Al assimilation in cowpea plants. These decreases in the Al contents can be explained by the formation of an Al–Si complex (Britez et al. 2002) in root apoplast, in the form of

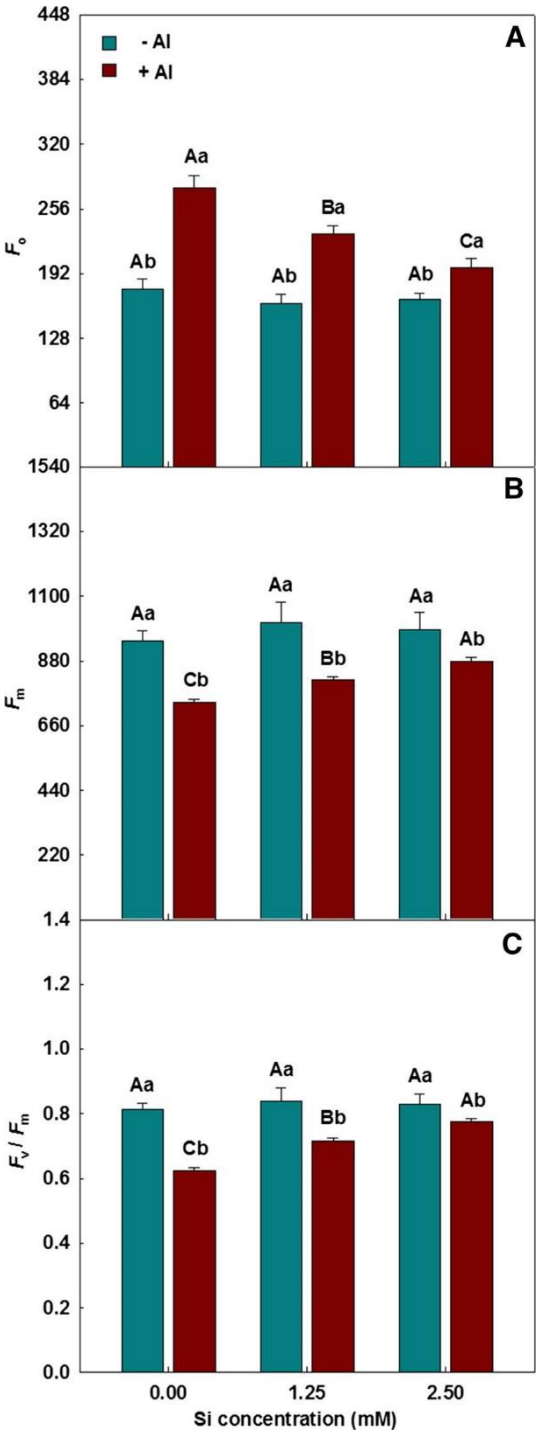

Fig. 1 Minimal fluorescence yield of the dark-adapted state (F_0), maximal fluorescence yield of the dark-adapted state (F_m) and maximal quantum yield of PSII photochemistry (F_v/F_m) in *V. unguiculata* plants treated with Si and exposed to Al toxicity. Different uppercase letters between Si levels (0, 1.25 and 2.50 mM Si under equal Al concentration) and lowercase letters between Al levels (0 and 10 mM Al under equal Si concentration) indicate significant differences from the Scott–Knott test ($P < 0.05$). Columns described corresponding to means from five repetitions and standard deviations

hydroxyaluminosilicate (Liang et al. 2007). This mechanism contributes to reducing the Al translocation of the root to other organs, such as leaves and stems. Wang et al. (2004), investigating the effect of Si in *Zea mays* seedlings subjected to Al toxicity, revealed hydroxyaluminosilicate formation in the root apoplast. Shen et al. (2014) observed reductions in the Al content in roots, stems and leaves of *Arachis hypogaea* seedlings when exposed to treatment with Si (Na_2SiO_3), corroborating the results in this research.

The exogenous application of Si induced increases in contents of all tissues, and these results reveal that Si was absorbed and translocated in the presence and absence of Al. The absorption of Si may occur by two mechanisms, active and/or passive diffusion (Ma and Yamaji 2008). Ma et al. (2004) studying Si uptake system in *Oryza sativa* identified Lsi1 and Lsi2 genes encoding the transporter proteins SIT1 and SIT2, respectively. Mitani and Ma (2005) reported that the Si transport via xylem differs according to the species, because it detects different densities of transporter proteins, with *Oryza sativa* > *Cucumis sativus* > *Lycopersicon esculentum*, in which the presence of SIT1 and SIT2 transporters was attributed to *Oryza sativa* and passive diffusion in *C. sativus* and *L. esculentum.*

Si contributed to increases in K, Ca, Mn, Fe and Zn in root, stem and leaf tissues, indicating also that Si mitigates the toxicity of Al, reducing the mineral imbalance. The availability of K is essential for gas exchange because the K^+ ions present in the guard cells are used in the stomatal mechanism. An increased Ca content is indicative of improvement in membrane integrity, reflected mainly in root elongation; however, reductions in Ca and Mg contents in plants exposed to Al can be attributed to competition with Al at the same sites of absorption in the roots (Ribeiro et al. 2013). The Mn, Fe and Zn elements are components of some enzymes involved in metabolic processes, such as respiration and photosynthesis, with Mn being the major constituent of the breakdown of the water molecule in PSII and Fe being a protein stabilizer. Giannakoula et al. (2008) showed reductions in K, Ca and Mg in the shoots (stem + leaf) and root of *Zea mays* lines exposed to different concentrations of $KAl(SO_4)_2$. Singh et al. (2011), investigating *O. sativa* seedlings, detected increases in Mn and Z contents in plants treated with 10 µM SiO_2 + 50 µM $AlCl_3$ by seven days. Both results corroborate our study.

Si mitigated the toxic effects of Al on F_0, F_m and F_v/F_m, confirmed by the decrease in F_0 and by the increases in F_m and F_v/F_m values. These results can be explained by increases in the Si concentrations that minimize the

Table 4 Chlorophyll fluorescence in *V. unguiculata* plants treated with Si and exposed to Al toxicity

Al (mM)	Si (mM)	Φ_{PSII}	q_P	NPQ	ETR (μmol m^{-2} s^{-1})	EXC (μmol m^{-2} s^{-1})	ETR/P_N
0	0	0.43 ± 0.03Aa	0.88 ± 0.08Aa	0.52 ± 0.05Ab	63.35 ± 4.2Aa	0.47 ± 0.03Ab	5.25 ± 0.40Ab
0	1.25	0.46 ± 0.02Aa	0.99 ± 0.09Aa	0.45 ± 0.04Ab	68.63 ± 3.3Aa	0.44 ± 0.03Ab	3.96 ± 0.35Bb
0	2.50	0.44 ± 0.02Aa	0.95 ± 0.04Aa	0.47 ± 0.04Ab	66.00 ± 2.4Aa	0.46 ± 0.02Ab	4.93 ± 0.24Ab
10	0	0.22 ± 0.02Cb	0.26 ± 0.04Bb	1.49 ± 0.05Aa	37.67 ± 2.4Bb	0.65 ± 0.03Aa	11.01 ± 0.49Aa
10	1.25	0.27 ± 0.02Bb	0.64 ± 0.11Ab	0.90 ± 0.05Ba	40.87 ± 4.0Bb	0.61 ± 0.02Aa	10.09 ± 0.38Ba
10	2.50	0.34 ± 0.01Ab	0.74 ± 0.09Ab	0.67 ± 0.03Ca	48.77 ± 1.1Ab	0.57 ± 0.01Ba	9.34 ± 0.70Ba

Columns with different uppercase letters between Si levels (0, 1.25 and 2.50 mM Si under equal Al concentration) and lowercase letters between Al levels (0 and 10 mM Al under equal Si concentration) indicate significant differences from the Scott–Knott test ($P<0.05$). Values described corresponding to means from five repetitions and standard deviations

Φ_{PSII}, effective quantum yield of PSII photochemistry; q_P, photochemical quenching coefficient; NPQ, nonphotochemical quenching; ETR, electron transport rate; EXC, relative energy excess at the PSII level; ETR/P_N, ratio between the electron transport rate and net photosynthetic rate

Table 5 Gas exchange in *V. unguiculata* plants treated with Si and exposed to Al toxicity

Al (mM)	Si (mM)	P_N (μmol m^2 s^{-1})	E (mmol m^{-2} s^{-1})	g_s (mol m^{-2} s^{-1})	C_i (μmol mol^{-1})	WUE (μmol $mmol^{-1}$)	P_N/C_i (μmol m^{-2} $s^{-1}Pa^{-1}$)
0	0	12.14 ± 0.49Ca	2.26 ± 0.21Ba	0.22 ± 0.009Ca	247 ± 20Ab	5.38 ± 0.11Ba	0.049 ± 0.001Ca
0	1.25	17.41 ± 0.71Aa	2.89 ± 0.21Aa	0.30 ± 0.025Aa	242 ± 10Ab	6.03 ± 0.25Aa	0.072 ± 0.007Aa
0	2.50	13.41 ± 0.55Ba	2.46 ± 0.17Ba	0.25 ± 0.009Ba	244 ± 12Aa	5.45 ± 0.19Ba	0.055 ± 0.002Ba
10	0	3.43 ± 0.30Bb	0.97 ± 0.08Ab	0.05 ± 0.015Ab	287 ± 15Aa	3.55 ± 0.22Cb	0.012 ± 0.002Bb
10	1.25	4.05 ± 0.42Bb	1.01 ± 0.10Ab	0.07 ± 0.008Ab	279 ± 11Aa	4.03 ± 0.15Bb	0.015 ± 0.001Bb
10	2.50	5.26 ± 0.49Ab	1.12 ± 0.11Ab	0.07 ± 0.007Ab	258 ± 08Ba	4.68 ± 0.24Ab	0.020 ± 0.002Ab

Columns with different uppercase letters between Si levels (0, 1.25 and 2.50 mM Si under equal Al concentration) and lowercase letters between Al levels (0 and 10 mM Al under equal Si concentration) indicate significant differences from the Scott–Knott test ($P < 0.05$). Values described corresponding to means from five repetitions and standard deviations

P_N, net photosynthetic rate; E, transpiration rate; g_s, stomatal conductance; C_i, intercellular CO_2 concentration; WUE, water-use efficiency; P_N/C_i, carboxylation instantaneous efficiency

damage caused by Al on the ultrastructure of chloroplasts, more specifically the PSII reaction centre. The increases in F_v/F_m detected in plants treated with Si demonstrate the reduction in photoinhibition and suggests increases in the photosynthetic activity in the reaction centre. Ribeiro et al. (2013) described a reduction of 14% in F_m after the increases in the $Al_2(SO_4)_3$ concentrations in the Catongo variety of *Theobroma cacao*.

The benefits on F_0 and F_m linked to Si action con- tributed to the increases in the Φ_{PSII}, q_P and ETR values. Si provided an increase in photon capture, inducing increases in the excitation energy absorption to PSII centres aimed at photochemical reactions, in addition to increasing the efficiency of plastoquinone and electron transport rates through the photosystems (Dallagnol et al. 2015).

Plants exposed to Al + Si presented reductions in NPQ, EXC and ETR/P_N values, suggesting that Si reduced thermal dissipation caused by the excessive excitation of the light-harvesting complex II, as well as reduced the alternative drains of electrons and consequently minimized Mehler reactions and the process of photorespiration. In other words, Si provided a better use of the electrons to photochemical activity.

Si promoted increases in P_N, E, and g_s values, alleviating the negative effects caused by Al in *V. unguiculata*, with this effect on the P_N connected to increases in PSII efficiency (Φ_{PSII}) and benefits in gas exchange (E and g_s). Higher values of E after the treatment with Si can be attributed to increases in g_s, suggesting an increase in tension into xylem to water and nutrient uptake (Zhang et al. 2013). Si has mitigated the changes caused by Al on the K^+/Ca^{2+}ions in guard cells, contributing positively to the stomatal mechanism, supported by increases in g_s.

Si mitigated the negative effects produced by Al in C_i due to Al neutralization and improvement in the efficiency of RUBISCO activity. Zhou et al. (2009), evaluating *Lycopersicon esculentum* cotyledons of under Al toxicity, detected alterations in two subunits that contribute to

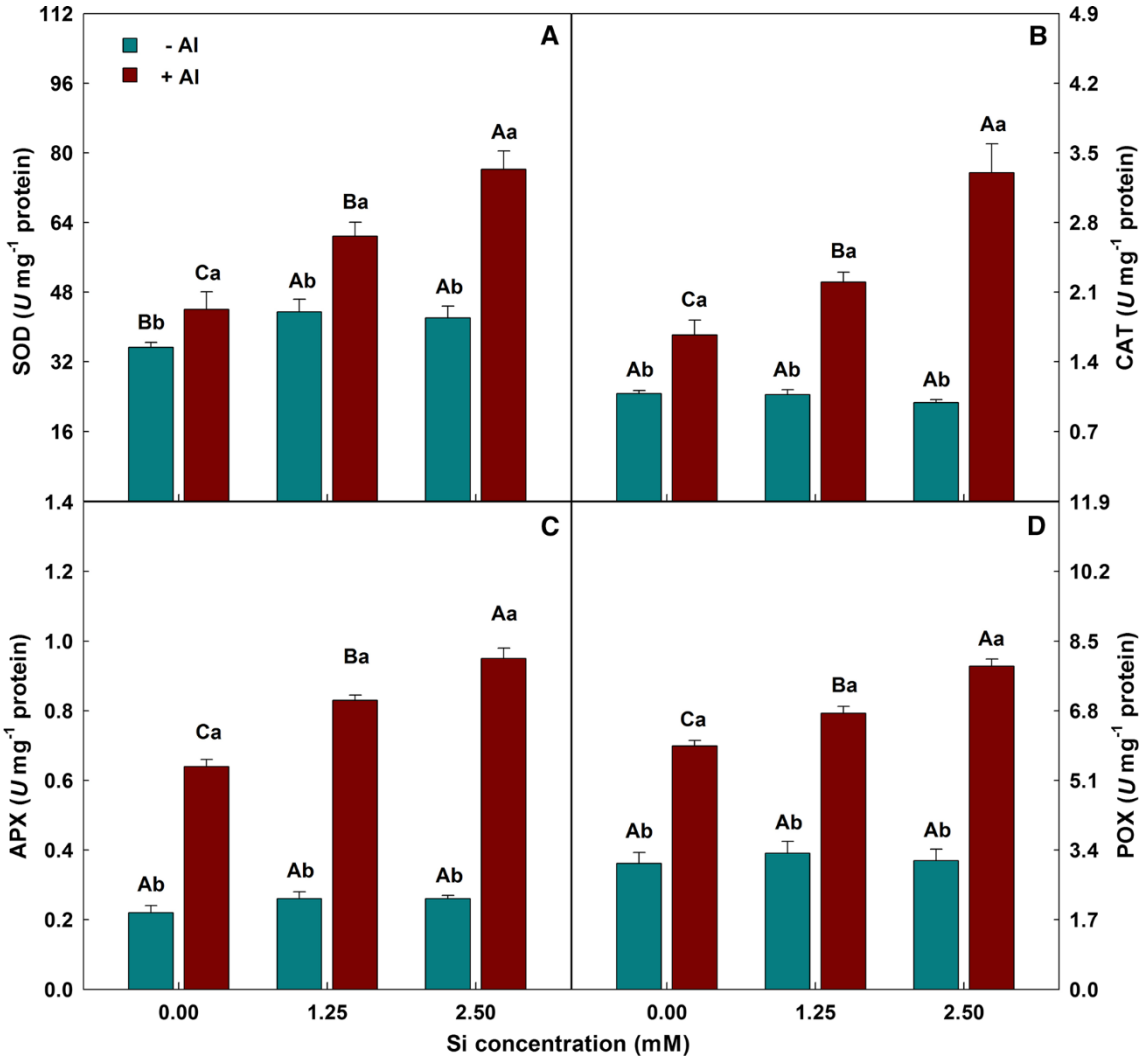

Fig. 2 Activities of superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX) and peroxidase (POX) in *V. unguiculata* plants treated with Si and exposed to Al toxicity. Different uppercase letters between Si levels (0, 1.25 and 2.50 mM Si under equal Al concentration) and *lowercase letters* between Al levels (0 and 10 mM Al under equal Si concentration) indicate significant differences from the Scott–Knott test ($P < 0.05$). Columns described corresponding to means from five repetitions and standard deviations

RUBISCO activity, being the main enzyme involved in the CO_2 assimilation process. Plants treated with Al had lower WUE values mainly caused by stomatal limitations, but Si induced a benefit on g_s, increasing P_N and E, and consequently increasing WUE, as parameter that is an important growth indicator in plants (Yang et al. 2015). Si application promoted increases in P_N/C_i values in plants exposed to Al, and it can be explained by increases in P_N and reductions in C_i values.

Plants treated with Al and Si had significant increases in the activities of SOD, CAT, APX and POX, revealing the positive action of Si in mitigating the oxidative damage to photosystems and membranes, supported by reductions in NPQ and EXC and increases in ETR obtained in this study after treatment with Si. Ali et al. (2011) reported increases in the activities of SOD, CAT, APX and POX in two *Hordeum vulgare* genotypes exposed to 100 µM of $AlCl_3$ at pH 4.0 in relation to the control treatment, corroborating our results.

Plants exposed to Si + Al presented decreases in O_2^- and H_2O_2 concentrations. The reduction of these compounds can be attributed to increases in the activities of antioxidant enzymes, which works to reduce ROS accumulation in chloroplast and mitochondria. SOD is the enzyme responsible for catalysing the radical O_2^- to H_2O_2 (Dong et al. 2002), and CAT, APX and POX are responsible for dismutating the H_2O_2 to form H_2O and O_2, which are enzymes of fundamental importance for the detoxification of plants caused by ROS (Gill and Tuteja 2010).

Si alleviated the damage caused by MDA and EL in plants exposed to Al toxicity, being explained by the lower concentrations of O_2^- and H_2O_2, indicating reductions in

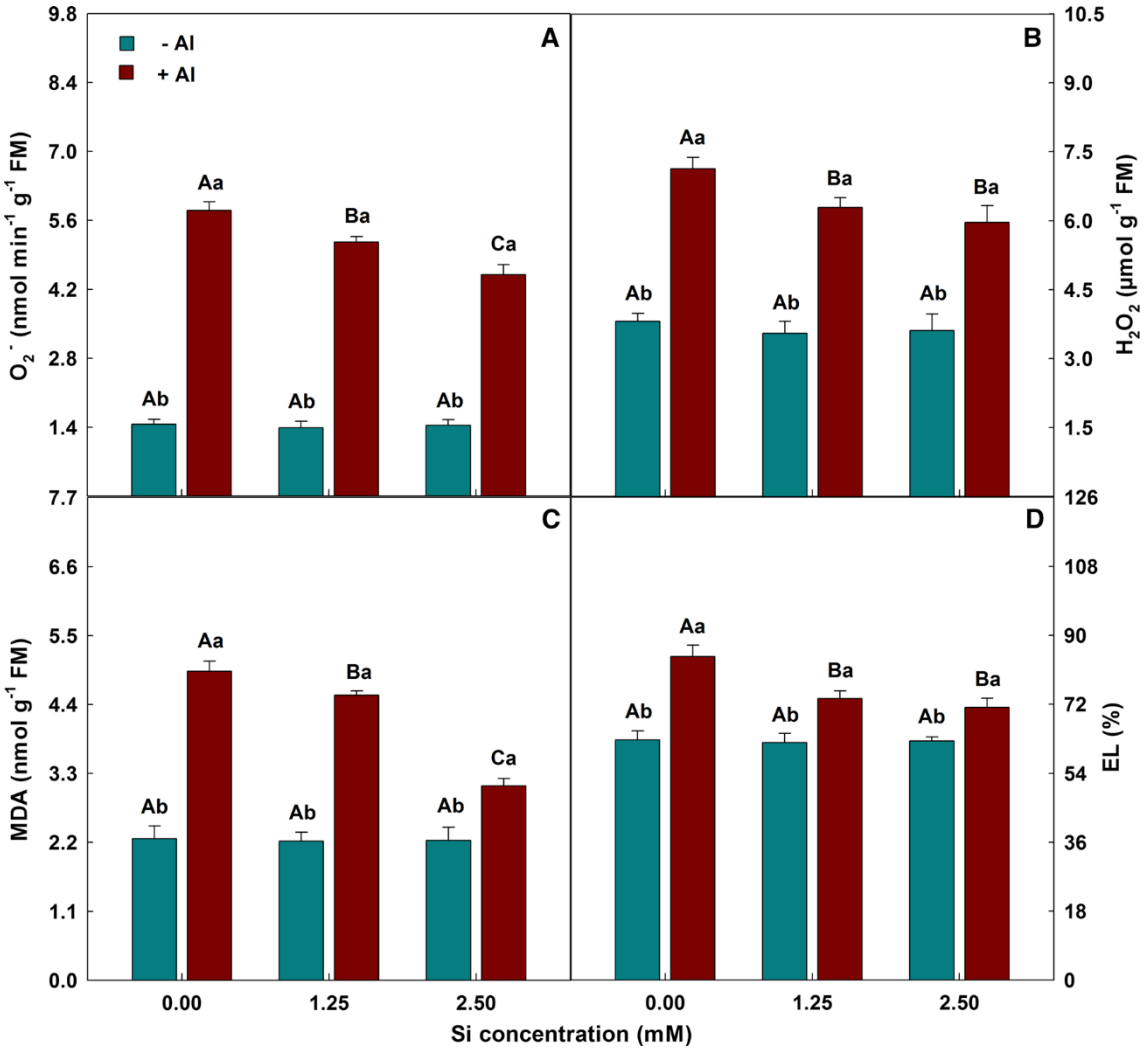

Fig. 3 Superoxide (O_2^-), hydrogen peroxide (H_2O_2), malondialdehyde (MDA) and electrolyte leakage (EL) in V. unguiculata plants treated with Si and exposed to Al toxicity. Different uppercase letters between Si levels (0, 1.25 and 2.50 mM Si under equal Al concentration) and *lowercase letters* between Al levels (0 and 10 mM Al under equal Si concentration) indicate significant differences from the Scott–Knott test ($P < 0.05$). Columns described corresponding to means from five repetitions and standard deviations

lipid peroxidation and higher membrane permeability. These parameters are used to indicate oxidative stress and describe the possible cell damage (Matsumoto and Motoda 2013). Shen et al. (2014) observed decreases in MDA and EL in *Arachis hypogaea* seedlings when subjected to a treatment of Al + Si and confirmed the beneficial effect of the Si in mitigating the oxidative damage caused by Al.

Plants exposed to Al toxicity + Si (2.50 mM) showed increases in Chl *a*, Chl *b*, total Chl and CAR, compared to control with 10 mM Al. This effect is associated with lower ROS concentrations in leaf tissue, confirmed in this study by O_2^- and H_2O_2. In addition to the reduction of the oxidative damage, increases in the levels of photosynthetic pigments may also be attributed to increases in the absorption and translocation of Mg, because this element is a component of the chlorophyll molecule (Yang et al. 2015). These benefits occasioned by Si improve the biosynthesis of pigments and promote a positive impact on the photosynthetic apparatus. He et al. (2011) observed reductions in total Chl investigating varieties of *Camellia oleifera* exposed to Al. Li et al. (2015) reported increases in Chl *a*, Chl *b*, total Chl and Car in *O. sativa* plants exposed to Mn + Si, which is consistent with results found in this research, emphasizing the beneficial action of Si in reducing the toxicity of metals.

The action of Si reduced the toxic effects of Al, such as increasing the photochemical efficiency of PSII, decreasing the negative effects on gas exchange, lowering the accumulation of oxidative compounds and increasing pigments. These benefits had positive repercussions on growth (RDM, SDM, LDM and TDM). The growth inhibition caused by Al is frequently linked to reductions in

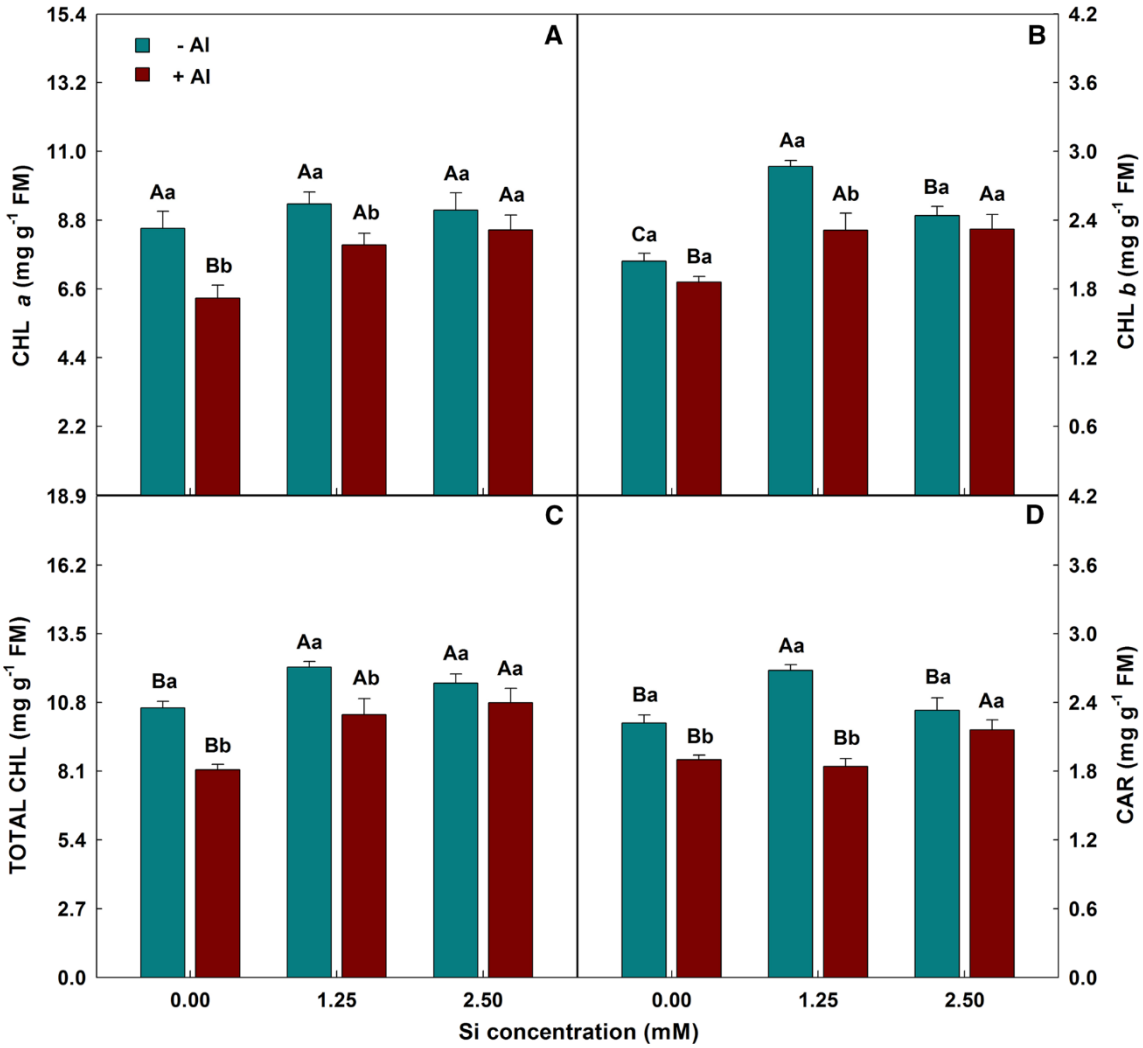

Fig. 4 Chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*), total chlorophyll (total Chl) and carotenoids (Car) in *V. unguiculata* plants treated with Si and exposed to Al toxicity. Different uppercase letters between Si levels (0, 1.25 and 2.50 mM Si under equal Al concentration) and lowercase letters between Al levels (0 and 10 mM Al under equal Si concentration) indicate significant differences from the Scott–Knott test ($P < 0.05$). Columns described corresponding to means from five repetitions and standard deviations

Table 6 Growth in *V. unguiculata* plants treated with Si and exposed to Al toxicity

Al (mM)	Si (mM)	LDM (g)	RDM (g)	SDM (g)	TDM (g)
0	0	8.45 ± 0.14Ba	5.83 ± 0.07Ca	5.99 ± 0.28Ca	20.27 ± 0.32Ca
0	1.25	9.32 ± 0.08Aa	10.32 ± 0.12Aa	7.16 ± 0.12Aa	26.80 ± 0.12Aa
0	2.50	9.21 ± 0.14Aa	7.2 ± 0.07Ba	6.68 ± 0.14Ba	23.09 ± 0.07Ba
10	0	6.23 ± 0.07Cb	4.37 ± 0.11Cb	4.70 ± 0.18Cb	15.30 ± 0.15Cb
10	1.25	6.50 ± 0.14Bb	4.99 ± 0.12Bb	5.05 ± 0.12Bb	16.54 ± 0.26Bb
10	2.50	7.16 ± 0.12Ab	5.68 ± 0.14Ab	5.84 ± 0.28Ab	18.68 ± 0.32Ab

Columns with different uppercase letters between Si levels (0, 1.25 and 2.50 mM Si under equal Al concentration) and lowercase letters between Al levels (0 and 10 mM Al under equal Si concentration) indicate significant differences from the Scott–Knott test ($P < 0.05$). Values described corresponding to means from five repetitions and standard deviations

LDM, leaf dry matter; RDM, root dry matter; SDM, stem dry matter; TDM, total dry matter

photosynthetic capacity and lower root development. Additionally, the increase in the photosynthetic rate promoted by Si contributed positively to increases in TDM. Dorneles et al. (2016) reported increase in the root dry matter of Solanum tuberosum plants subjected to 1.0 mM Na_2SiO_3 + 1.85 mM $AlCl_3$. Mali and Aery (2008) detected increases in dry matter shoots (stem + leaf) and roots in *V. unguiculata* plants exposed to 100 µg Si g^{-1} soil. Both results corroborate our findings.

Conclusions

Our results proved that Si reduced the Al contents in all tissues. Si also improved the photochemical efficiency of PSII, gas exchange, pigments and antioxidant enzymes, contributing to reducing the accumulation of oxidative compounds. These benefits corroborate the multiple roles exercised by Si on metabolism, in addition to revealing that Si immobilizes the Al in roots and reduces the accumulation of this metal in other organs, mitigating the damage caused by Al in cowpea plants. In relation to dose–response, plants under 1.25 mM Si without Al presented better results for growth, whereas plants exposed to Al toxicity mitigated the toxic effects with 2.50 mM Si.

Author contribution statement LAKS was advisor of this project, planning all phases of this research. JLR conducted the experiment in the greenhouse and performed physiological, biochemical and morphological determinations. BBL carried out nutritional determinations and helped in drafting the manuscript and in interpreting the results.

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