Comissão 3.5 - Poluição, remediação do solo e recuperação de áreas degradadas

EFFECTS OF SILICON ON ALLEVIATING ARSENIC TOXICITY IN MAIZE PLANTS

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ABSTRACT

Arsenic is a metalloid highly toxic to plants and animals, causing reduced plant growth and various health problems for humans and animals. Silicon, however, has excelled in alleviating stress caused by toxic elements in plants. The aim of this study was to investigate the effects of Si in alleviating As stress in maize plants grown in a nutrient solution and evaluate the potential of the spectral emission parameters and the red fluorescence (Fr) and far-red fluorescence (FFr) ratio obtained in analysis of chlorophyll fluorescence in determination of this interaction. An experiment was carried out in a nutrient solution containing a toxic rate of As (68 µmol L^{-1}) and six increasing rates of Si (0, 0.25, 0.5, 1.0, 1.5, and 2.0 mmol L^{-1}). Dry matter production and concentrations of As, Si, and photosynthetic pigments were then evaluated. Chlorophyll fluorescence was also measured throughout plant growth. Si has positive effects in alleviating As stress in maize plants, evidenced by the increase in photosynthetic pigments. Silicon application resulted in higher As levels in plant tissue; therefore, using Si for soil phytoremediation may be a promising choice. Chlorophyll fluorescence analysis proved to be a sensitive tool, and it can be successfully used in the study of the ameliorating effects of Si in plant protection, with the Fr/FFr ratio as the variable recommended for identification of temporal changes in plants.

Index terms: heavy metals, metalloids, photosynthetic pigments, trace element.

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RESUMO: *EFEITOS DO SILÍCIO NO ALÍVIO DA TOXICIDADE DE ARSÊNIO EM PLANTAS DE MILHO*

Arsênio (As) é um metaloide de grande potencial de toxidez para plantas e animais, causando redução do crescimento vegetal e diversos problemas de saúde humana e animal. O silício (Si), por sua vez, tem se destacado na amenização de estresses causados por elementos tóxicos em plantas. Os objetivos deste trabalho foram investigar os efeitos do Si na amenização do estresse causado por As em plantas de milho cultivadas em solução nutritiva e avaliar o potencial dos parâmetros de emissão espectral e razão Fr/FFr (comprimento de onda vermelho/vermelho distante), obtidos na análise da fluorescência da clorofila na determinação dessa interação. Para isso, foi montado um experimento em solução nutritiva com uma dose tóxica de As (68 µmol L^{-1} de As) e seis doses crescentes de Si (0; 0,25; 0,5; 1,0; 1,5; e 2,0 mmol L^{-1} de Si), sendo avaliados produção de matéria seca, teores de As, Si e pigmentos fotossintéticos. Medidas de fluorescência da clorofila foram tomadas ao longo do cultivo. O silício promove efeitos positivos na amenização do estresse causado por As em plantas de milho comprovado pelo incremento de pigmentos fotossintéticos. A aplicação de Si proporcionou maiores teores de As no tecido vegetal e a sua utilização em estudos de técnicas de fitorremediação de solos contaminados pode ser promissora. A análise de fluorescência da clorofila demonstrou ser uma ferramenta sensível, podendo ser empregada com sucesso no estudo dos efeitos amenizantes do Si na proteção de plantas, sendo a razão Fr/FFr a variável recomendada na identificação de alterações temporais em plantas.

Palavras-chave: metais pesados, metaloides, pigmentos fotossintéticos, elementos traços.

INTRODUCTION

Millions of people around the world are exposed to As. A classic case is groundwater contamination in Bangladesh, which is a significant public health problem affecting 35 to 75 million people (Chen et al., 2009). Arsenic is one of the most carcinogenic elements to humans, with involvement in skin, lung, bladder, liver, and kidney cancers. It is also linked to several other adverse health effects (Melkonian et al., 2010; Ahsan and Steinmaus, 2013); diabetes, cardiovascular diseases, pre-natal complications, and decreased intellectual function in children are also related to As exposure (Chen et al., 2009). The risk is greater for people living near mining areas, where exposure of plants and animals to As is high. The concentration of As in people's hair and urine (2.92 mg kg^{-1} and 164 µg L^{-1}, respectively) in these areas suggests health damage to the populations (Liu et al., 2010).

Silicon has been recognized as a toxicity alleviating agent in plants through retaining heavy metals in roots, inhibiting their translocation to the shoots (Shi et al., 2005a), depositing SiO_2 in the apoplast of the roots and on the leaf surface, forming a barrier to the apoplastic flow of metallic ions and to transpiration flux (Lux et al., 2002; Shi et al., 2005a). It also contributes to the co-precipitation of Si-metal complexes in the cell wall, compartmentalization of metals bound to organic acids in the vacuole (Neumann and Nieden, 2001), more homogeneous distribution of metals, formation of Si-polyphenol complexes in tissues (Maksimovic et al., 2007), and reduced lipid peroxidation in the membrane, via stimulation of enzymatic and non-enzymatic antioxidants (Shi et al., 2005b). Silicon is linked to lower As translocation to rice straw and grains (Ali et al., 2009), with proven benefits in food safety. Increased production of antioxidant enzymes, reduced lipid peroxidation, and reduced As levels in rice plants were observed when Si was added to the growth solution (Tripathi et al., 2013).

Chlorophyll fluorescence emission by the leaves is altered when biotic and abiotic stresses cause disruption to the photosynthetic apparatus, either directly or indirectly (Baker and Rosenqvist, 2004). The chlorophyll fluorescence technique, in addition to being nondestructive, is highly sensitive and simple to use, generating qualitative and quantitative information about the physiological status of the photosynthetic apparatus of plants (Falqueto et al., 2007). Many parameters can be obtained from chlorophyll fluorescence analysis. Several studies have shown the ratio between the emission peaks in the red (F685) and far red (F735) regions as a sensitive variable for identification of stress caused by heavy metals in plants (Cherif et al., 2009; 2010).

Chlorophyll fluorescence analysis has proven to be a promising tool for detecting Cd toxicity in maize plants (Silva et al., 2012). Variations in the emission peaks of chlorophyll fluorescence and their respective ratios were effective in detecting Cd-related stress in maize (Maurya et al., 2008). The technique was also effective in analysis of As toxicity in maize (Stoeval and Bineva, 2003).

Chlorophyll fluorescence analysis has also shown significant results in identifying Si benefits to plant nutrition, especially when under abiotic or biotic stress. For example, Si has led to beneficial effects (such as increased quantum yield and effective maximum quantum) on the photosynthesis of cucumber plants grown in the presence of Cd (Feng et al., 2010). Silicon was also effective in alleviating Cr stress by increasing the concentrations of photosynthetic pigments and the efficiency of chlorophyll fluorescence parameters (Ali et al., 2013). Silicon application increased quantum efficiency of open PS2 centers in a dark-adapted state (Fv/Fm) and photochemical quenching coefficient in a light-adapted state (qP) parameters in rice plants under Cd stress (Nwugo and Huerta, 2008a).

The aim of this study was to investigate the effects of Si in alleviating As stress in maize plants grown in a nutrient solution. An additional aim was to evaluate the potential of spectral emission parameters and the Fr/FFr ratio obtained in the analysis of chlorophyll fluorescence in determining the Si-As interaction in maize plants under a toxic level of As.

MATERIAL AND METHODS

The experiment was carried out in a greenhouse located at Agronomy Department of the UFRPE. Maize seeds (*Zea mays* L. 'São José') were germinated between sheets of paper towel, with the base immersed in a 0.67 mmol L^{-1} $Ca(NO_3)_2.4H_2O$ solution (Vilela and Anghinoni, 1984). Seven days after sowing, two seedlings were transferred to a plastic container with 6 L of a [modified] nutrient solution (Hoagland and Arnon, 1950), containing 105.05 mg L^{-1} N, 15.5 mg L^{-1} P, 117.3 mg L^{-1} K, 100.2 mg L^{-1} Ca, 24.3 mg L^{-1} Mg, 32.1 mg L^{-1} S, 0.65 mg L^{-1} Cl, 0.5 mg L^{-1} Mn, 0.05 mg L^{-1} Zn, 0.02 mg L^{-1} Cu, 0.5 L^{-1} B, 0.01 mg L^{-1} Mo, and 7.53 mg L^{-1} Fe. The nutrient solution was replaced weekly and/or whenever electric conductivity reached 0.4 dS m^{-1}. Deionized water was added to the pot to replace the water lost by evapotranspiration. The pH was maintained close to 5.50±0.2 and adjustments were made with 1 mmol L^{-1} H_2SO_4 or NaOH.

After 12 days for plant adaptation to the nutrient solution, the toxic As level obtained in the previous experiment (68 μmol L^{-1}) and the Si (K_2SiO_3) rates of 0, 0.25, 0.5, 1.0, 1.5, and 2.0 mmol L^{-1} were added to the solution. Maize plants were then grown for 21 days.

Five chlorophyll fluorescence measurements were carried out during the experiment. The first was taken before As addition and the last was carried out one day before collection. Measurements were taken at night to ensure deactivation of electron transport in the photosynthetic apparatus. In vivo chlorophyll fluorescence analyses were conducted with an ultraviolet LED device, with peaks at the 685 and 735 ηm wavelengths. The spectra were obtained by the Ocean Optics Spectra-Suite software and adjusted to two Gaussian curves corresponding to red (685 ηm) and far red (735 ηm). The ratio between the F685/F735 fluorescence intensity and peak height was calculated from the curve fitted to each Si application rate and used to infer the effect of the element in the photosystem II, using the Origin software version 6.0.

Leaves were sampled to determine the levels of chlorophyll a and b and, through the sum, the total chlorophyll content (Arnon, 1949), with the middle third of the same leaf used for chlorophyll fluorescence analysis.

Leaves, stems, and roots were rinsed with tap water, followed by three rinses with distilled water before being placed in paper bags. The samples were then placed in an air circulation laboratory oven at 65 °C until reaching constant weight so as to obtain the dry matter of leaves, stems, and roots, as well as total dry matter.

Digestion of plant material was performed in nitric and hydrochloric acids in a microwave oven, according to the 3051A Method (USEPA, 1998). Arsenic content was determined from the digestion extract in an atomic absorption spectrophotometer coupled to a hydride generator. Silicon digestion in the plant tissue was carried out with hydrogen peroxide (H_2O_2) and sodium hydroxide (NaOH) in an autoclave. Rates were determined in a colorimeter using ammonium molybdate as a complexing agent (Korndörfer et al., 2004). The data originating from four replicates were subjected to ANOVA and regression analyses.

RESULTS AND DISCUSSION

Dry matter production

The production of dry matter from leaves and roots was not affected by the increasing Si rates (Table 1); a significant effect was only observed for the stem and total dry matter. Si accumulation in plants does not always have positive effects on dry matter production (Melo et al., 2009; Araújo et al., 2011), but it can bring other beneficial effects, such as protection against physiological stress by improving the photosynthetic apparatus (Mattson and Leatherwood, 2010).

Silicon and Arsenic accumulation in plants

Maize plants responded positively to Si application in the nutrient solution (Figure 1). An Si content greater than 1 % in the shoots

Table 1. Dry matter production of maize plants under As stress and Si application rates

Silicon rate	Leaf^{ns}	Stem*	Root^{ns}	Total*
mmol L^{-1}		g/pot		
0.00	34.97	30.72	22.99	88.69
0.25	29.64	24.19	17.97	71.79
0.50	30.87	28.04	20.73	79.64
1.00	30.08	25.48	19.22	74.78
1.50	33.01	29.96	21.11	84.08
2.00	30.53	28.90	21.42	80.85

* significant at 5 %; ^{ns}: Non-significant.

Table 2. Arsenic content in leaves, stems, and roots of maize plants grown in nutrient solutions with Si

Si rate	Leaf**	Stem^{ns}	Root^{ns}
mmol L^{-1}		mg kg^{-1}	
0.00	0.66	1.37	75.22
0.25	0.81	1.73	88.89
0.50	1.07	1.92	93.78
1.00	1.20	2.13	99.78
1.50	0.89	1.92	91.95
2.00	0.93	1.69	91.03

** significant at 1 %; ^{ns}: Non-significant.

characterizes the species as a accumulator of the element (Ma et al., 2001). Furthermore, greater Si accumulation in the shoots (leaves and stems) indicates that maize plants have a mechanism of active transport for this element, similar to rice (Nwugo and Huerta, 2008b).

Silicon taken up by the plants is translocated to the shoots by water flow; in the leaves, concentration of the element is determined by water loss through the stomata. This causes Si polymerization in the apoplast of leaves, forming an important barrier to protect plants against various types of stress (Mitani et al., 2005) or causing co-precipitation of toxic elements. This can be observed for As in all plant parts (Table 2), which showed increased concentrations of the element up to 1 mmol L^{-1} Si in solution. Si addition was effective in alleviating As stress in rice plants, proving to be effective in reducing As levels in the shoots, with accumulation of antioxidant enzymes. Furthermore, Si addition increased the concentrations of cysteine and reduced lipid peroxidation (Tripathi et al., 2013). Silicon accumulators, such as rice, accumulate As at higher levels than many other species, because As and silicic acid share the same carrier (Chen et al., 2012).

A higher level of As in plants demonstrates the potential use of Si in soil remediation techniques, such as phytostabilization and phytoextraction. Interestingly, a reduction in tissue levels of As for the two highest doses was observed, showing an effect of decreased As uptake with high Si application rates.

The results imply that Si changes the forms of As taken up and accumulated in different plant parts. Vegetative growth is not affected and no toxicity symptoms are observed, even with higher As content in the tissue. The various chemical forms of As may represent different toxicity levels (Jedynak et al., 2012). The biotransformation of As^{3+} into the less toxic As^{5+} through oxidation, mainly using Fe and sulfates, is one of the mechanisms activated in both prokaryotic and eukaryotic microorganisms (Halter et al., 2012).

Production of photosynthetic pigments

The addition of Si to the nutrient solution increased the levels of photosynthetic pigments (Table 3), proving the beneficial effects of this element in alleviating As stress. The chlorophyll a and total chlorophyll levels were altered, whereas chlorophyll b (accessory pigment) did not vary with Si addition. Photosynthetic pigments are highly responsible for photosynthetic metabolism in plants; chlorophyll a is the main agent responsible for allowing this interaction between the capture of solar energy and the process initiated within the chloroplasts, whereas chlorophyll b acts as an accessory pigment in the process of electron transfer to chlorophyll a (Caires et al., 2010; Bohr and Malik, 2011).

The results show that Si addition to the nutrient solution leads to a higher content of photosynthetic pigments for all Si rates applied, mainly for chlorophyll a and total chlorophyll; the highest levels for all pigments were found with 1 mmol L^{-1} Si.

Silicon was important in alleviating the visual symptoms of Mn toxicity in cucumber plants, reflecting an increase in the levels of photosynthetic pigments

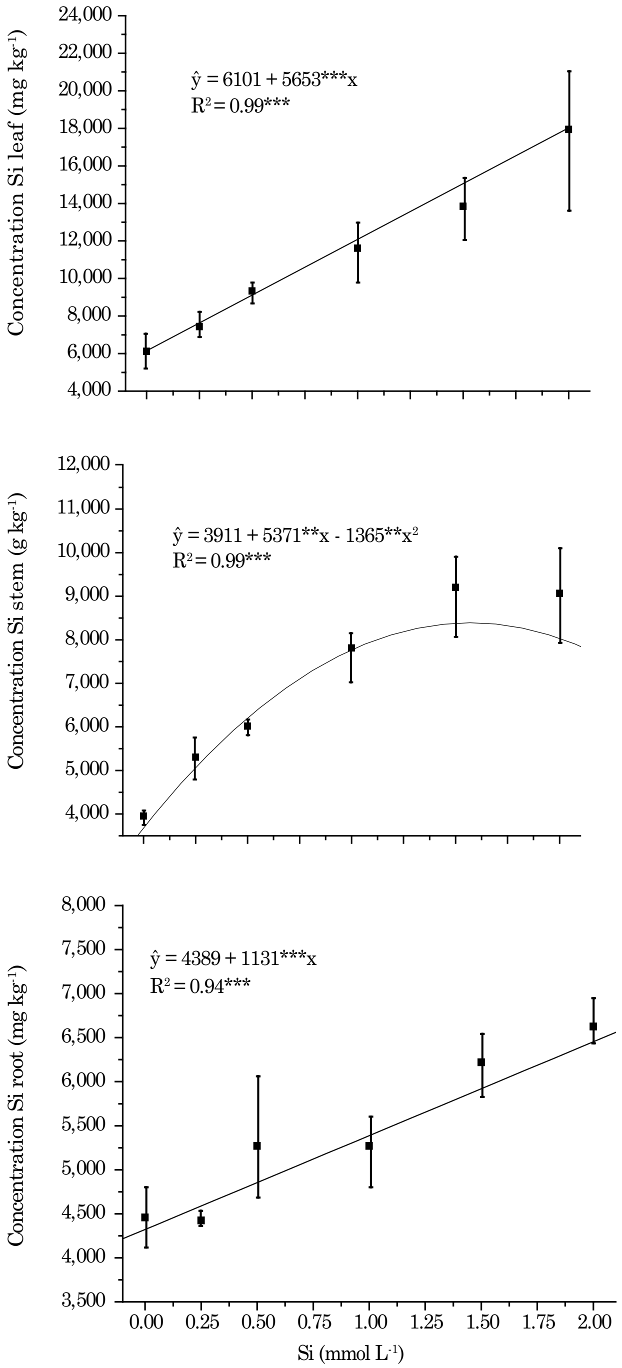

Figure 1. Silicon content in leaves, stems, and roots of maize plants grown in a nutrient solution contaminated by arsenic and under increasing application rates of Si. ** and *: significant at 1 and 0.1 %).**

Table 3. Photosynthetic pigment content as a function of silicon (Si) application rates in maize plants grown in a nutrient solution with arsenic

Si rate	Chlorophyll content		
	a*	b^{ns}	Total*
mmol L^{-1}		mg g^{-1}	
0.00	1.12	1.24	2.36
0.25	1.80	1.51	3.31
0.50	1.79	1.21	3.00
1.00	1.95	1.44	3.39
1.50	1.81	1.46	3.27
2.00	1.63	1.49	3.11

* significant at 5 %; ^{ns} non-significant.

when 1 mmol L^{-1} was applied (Feng et al., 2009). An increase in the content of photosynthetic pigments was also observed after Si application to cucumber plants under Cd stress (Feng et al., 2010). These authors observed that Si led to improvements in gas exchange, favoring lower Cd accumulation in leaves, reducing damage to chloroplasts, and protecting their ultrastructure. Reduced symptoms of As toxicity, even with higher levels of the element in leaves, was observed in this study, indicating that a decrease in As levels occurred where photosynthesis is most active, thus resulting in stress alleviation in leaf structures.

Chlorophyll fluorescence monitoring

Alleviation of As stress to the photosynthetic apparatus by Si application varied according to the rates applied. There was a reduction in the spectral emission at 1 mmol L^{-1} Si (Figure 2), even though this rate caused greater As accumulation in plant leaves (Table 2). It is observed that Si application rates greater than 1 mmol L^{-1} cause more stress than the presence of As, indicating the existence of an optimal dose for stress alleviation. This result corresponds to the best response of chlorophyll a and total chlorophyll (Table 3), corroborating these findings. An increase in photochemical efficiency and electron transport of photosystem II were caused by the addition of 1 mmol L^{-1} Si to cucumber plants grown under Mn stress (Feng et al., 2009).

Over time, this difference is much more explicit, showing the importance of the Fr/FFr ratio as the most sensitive parameter in identification of As stress and its alleviation by Si (Figure 3). The difference in the ratio can be observed in the reading taken at five days of growth under As and Si application rates. The reduction in the Fr/FFr ratio confirms the beneficial effects of Si to the photosynthetic apparatus of maize plants. As the control showed the highest Fr/FFr ratio, all Si application rates were effective in alleviating stress.

The effects of Si on the growth, photosynthesis, and chlorophyll a fluorescence parameters in plants under Cd stress play an important role in protection of the photosynthetic apparatus since Si application increases the quantum yield and the maximum

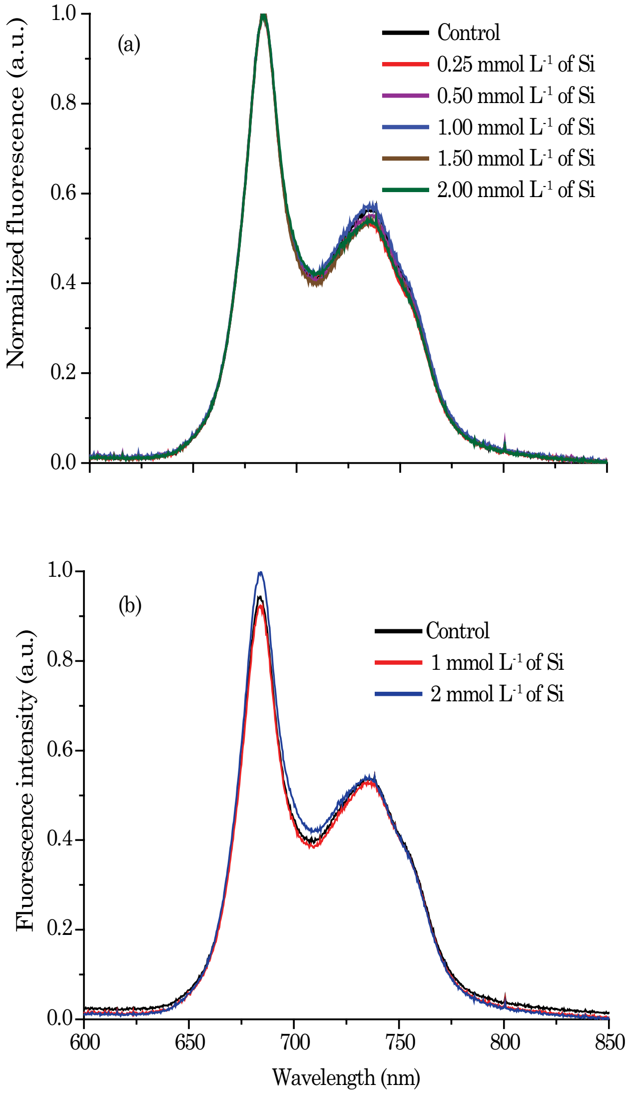

Figure 2. (a) Spectra of chlorophyll fluorescence in maize plants grown under different Si application rates in a nutrient solution contaminated by arsenic. (b) Maximum intensity of chlorophyll fluorescence for the highest silicon application rates in the nutrient solution.

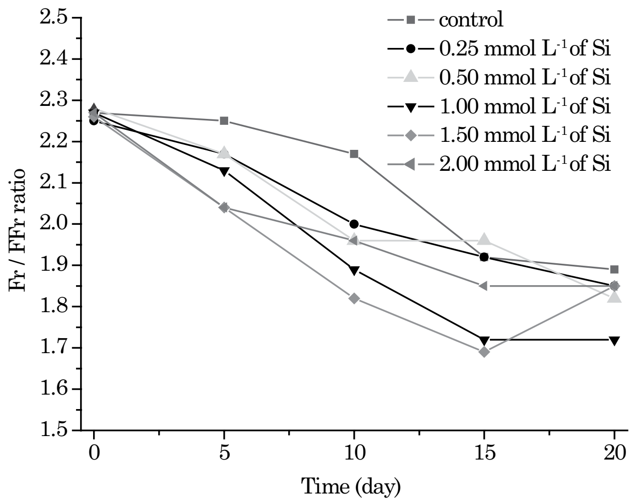

Figure 3. Spectra of the chlorophyll fluorescence ratio as a function of growing time of maize plants under increasing silicon application rates in a nutrient solution contaminated by arsenic (Fr/FFr at the F685 ηm and F735 ηm wavelengths, respectively).

effective quantum yield of the photosystem II (Feng et al., 2010). This result can be confirmed for As stress in maize plants.

Silicon was also effective in alleviating Cr toxicity and improving the photosynthetic parameters and the efficiency of chlorophyll fluorescence parameters. This is possible because Si has the ability to alleviate the damage caused to cell ultrastructure in leaves and roots (Ali et al., 2013). The analysis of chlorophyll fluorescence suggests that Si alleviates Cd toxicity in rice plants under low stress levels, as minimum chlorophyll fluorescence yield in a dark-adapted state (Fo) reduction and quantum efficiency of open PS II (photosystem II) centers in a dark-adapted state (Fv/Fm) and photochemical quenching coefficient in a light-adapted state (qP) increase have been observed, thus improving efficiency in the use of light (Nwego and Huerta, 2008a).

Morphological differences between maize leaves with and without supplemental Si showed different results in Mn alleviation. The increased thickness of the epidermal layers suggests an important role for these cells in Mn tolerance, both in genetic Mn tolerance and in Si-induced tolerance (Doncheva et al., 2009). In the presence of Si, Brassica plants showed more accelerated endoderm development, compared with plants grown only in the presence of Cd. This may have led to lower Cd uptake and reduced toxicity due to a suberin lamella formed in the endodermis of plants supplied with Si (Vatehová et al., 2012).

The results of this study confirm that Si is an important element in alleviating As stress in maize plants since Si provided greater efficiency in the photosynthetic parameters and an increase in photosynthetic pigment levels in plants.

CONCLUSIONS

The use of Si to alleviate As stress in maize plants grown in a nutrient solution showed positive results in protecting the photosynthetic apparatus.

Silicon alleviated the deleterious As effect, which resulted in a higher production of photosynthetic pigments in the leaves.

The analysis of chlorophyll fluorescence has proven to be a sensitive tool; thus the technique can be successfully employed in studying the effects of Si on alleviating toxicity in plants. This is possible due to the positive effects of Si in protecting the photosynthetic apparatus, with the Fr/FFr ratio being the variable recommended for identifying temporal changes in plants.

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