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Assessment of cadmium phytotoxicity alleviation by silicon using chlorophyll *a* fluorescence

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Abstract

The aim of this study was to investigate the effects of silicon in alleviating cadmium stress in maize plants grown in a nutrient solution and to evaluate the potential of the spectral emission parameters and the ratio of red fluorescence (Fr) to far-red fluorescence (Ffr) in assessing the beneficial effects of Si. An experiment was carried out using a nutrient solution with a toxic dose of Cd and six doses of Si; biomass, Cd, Si, and photosynthetic pigments of the plants were measured. Chlorophyll (Chl) *a* fluorescence analysis demonstrated that Si alleviated Cd toxicity in plants. Chl fluorescence measurements were sensitive in detecting such effects even when significant changes in biomass production and concentrations of photosynthetic pigments were not observed. The spectral emission and the Fr/Ffr ratio were sensitive to the effects of Si. Silicon caused a reduction in the translocation of Cd to the shoots of maize plants.

Additional key words: metal homeostasis; metal toxicity; soil contamination; soil pollution.

Introduction

Cadmium is one of the most widespread and toxic metals in soils. It is mainly produced by industrial activities, mining, and zinc refining and has been reported to be the metal with the highest rate of global emission into soils (Alloway 1990). The entry of Cd into agricultural soils takes place through the application of phosphate fertilizers, sewage sludge, manure, and liming (Freitas *et al.* 2009). Cd can alter the biosynthesis of Chl in plants by inhibiting a reductase enzyme as well as PSII electron transport (Lagriffoul *et al.* 1998, Moradi and Ehsanzadeh 2015), and by causing nutritional imbalance in the plant, especially on PSII electron donor side (Nascimento *et al.* 1998, Costa *et al.* 2012, Li *et al.* 2012). Bazzaz and Govindjee (1974) observed that PSII activities, including fluorescence yield of Chl *a*, are inhibited strongly by Cd; they also reported that Cd causes degradation of Chl and carotenoids, inhibiting their biosynthesis and inducing oxidative stress.

High concentration of Si in the plant tissue can promote Si-Cd coprecipitation (Gu *et al.* 2011). Additionally, fertilizing maize plants with Si results in an increase of crop yields which, in turn, further reduces abiotic stress due to dilution effects (Kim *et al.* 2011, Suriyaprabha *et al.* 2012). Zinc, Mn, Cu, and Ni concentrations are highly correlated with Si concentrations in the trichomes, indicating a possible coprecipitation effect; the presence of SiO_2 in intercellular spaces further provides evidence for the stabilization of metal silicates when Si is used to alleviate the toxicity of these metals. Si has also been associated with alleviation of toxicity caused by Cd, Zn, and Mn in *Picris divaricate* (Broadhurst *et al.* 2013).

The main internal mechanism of Si-mediated stress reduction in plants is metals and Si co-deposition within plants, inhibition of root-shoot transport of metals, compartmentalization of metals in vacuoles, increased binding of metals to cell walls with decreased concentrations of metals in the symplast, and reduction of membrane lipid peroxidation *via* stimulating enzymatic and nonenzymatic reactions (Liang *et al.* 2007, Cunha and Nascimento 2009). The mechanism of Si alleviation of metal toxicity includes reduction of metal solubility *via* Si-increased ionic strength or pH, Si-mediated release of phenolic compounds, and metal and Si coprecipitation either in different parts of the plant or in the growth media (Liang *et al.* 2007).

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Abbreviations: Chl – chlorophyll; ChlF – Chl fluorescence; Fr – red fluorescence; Ffr – far-red fluorescence; LED – light emitting diode; F_v – variable Chl *a* fluorescence; F_m – maximum Chl *a* fluorescence; F_o – minimal Chl *a* fluorescence; q_P – coefficient of photochemical quenching.

Because of changes induced by Cd in the photosynthetic apparatus of plants, Chl *a* fluorescence (ChlF) analysis can be used to detect stress induced by Cd toxicity (Bazzaz and Govindjee 1974, Silva *et al.* 2012). This technique is useful in monitoring the beneficial effects produced by Si during the alleviation of heavy metal toxicity (Feng *et al.* 2010); it represents a very important tool for evaluating changes in photosynthetic pigments, as it provides information on the photochemical activity of PSII, changes in photosynthetic pigments, primary light reactions, electron transport in thylakoids, enzymatic reactions, which occur in the stroma, and the slow processes of metabolic regulation in plants (*see e.g.*, Papageorgiou and Govindjee 2004, Kalaji and Guo 2008).

Silicon has positive effects on photosynthesis of plants under stress; for example, Si treatment is known to increase the quantum yield and maximum effective quantum yield of photosynthesis in cucumber plants grown in the presence of Cd (Feng *et al.* 2010). Si is also able to alleviate Cr stress, increasing the concentration of photosynthetic pigments and changing specific ChlF parameters (Ali *et al.* 2013). The application of Si is known to increase the F_v/F_m (variable, F_v, to maximum, F_m, Chl *a* fluorescence, where $F_v = F_m - F_o$) and the photochemical quenching (q_P) parameter of rice plants grown under Cd stress (Nwugo and Huerta 2008).

Several studies have shown that the ratio between the emission peaks at the red (F_{685}) and far red (F_{735}) region is sensitive to stress caused by metals in plants (Cherif *et al.* 2010, 2012; Silva *et al.* 2012, Marques and Nascimento 2013). Measurements, which are needed to characterize metal stress in plants using ChlF, can be made quickly and easily; furthermore, this technique is noninvasive, nondestructive and simple to use (Papageorgiou and Govindjee 2004, Kalaji and Guo 2008, Woo *et al.* 2008, Marques and Nascimento 2014, Kumar and Prasad 2015). Thus, this study was carried out to assess the ability of Si to alleviate Cd toxicity in maize plants grown in a Cd-contaminated nutrient solution. Taking into account the beneficial effects of Si on photosynthesis of plants under metal stress, we also measured emission spectra and the Fr/Ffr ratio obtained from the ChlF analysis for assessing the Si-mediated phytotoxicity amelioration.

Materials and methods

Plant material and greenhouse experiment: Maize seeds (*Zea mays* L., cv. São José) were germinated on layers of paper towels with the bottom layer immersed in a solution containing 0.67 mmol L^{-1} Ca in the form of $Ca(NO_3)_2 \times 4\,H_2O$. Seven days after sowing, two seedlings were transferred to a plastic pot containing 6 liters of a modified nutrient solution, as described by Hoagland and Arnon (1950), containing 105.05 mg(N) L^{-1}, 15.5 mg(P) L^{-1}, 117.3 mg(K) L^{-1}, 100.2 mg(Ca) L^{-1}, 24.3 mg(Mg) L^{-1}, 32.1 mg(S) L^{-1}, 0.325 mg(Cl) L^{-1}, 0.25 mg(Mn) L^{-1}, 0.025 mg(Zn) L^{-1}, 0.01 mg(Cu) L^{-1}, 0.25 mg(B) L^{-1}, 0.005 mg(Mo) L^{-1}, and 7.53 mg(Fe) L^{-1}. The nutrient solution was replaced once a week and/or when the electrical conductivity reached 0.4 dS m^{-1}. Deionized water was added to the pots to replace the water lost by evapotranspiration. The pH was maintained close to 5.50 (± 0.2) and adjusted with 1 mmol L^{-1} solution of H_2SO_4 or NaOH. Plants were grown in a naturally illuminated greenhouse. Average temperature and relative humidity during plant growth were 28ºC and 79%, respectively.

After a period of eight days, during which the plants were allowed to adapt to the nutrient solution, a toxic concentration of Cd (30 µmol L^{-1}) was added to the solution; this dose was chosen based on a previous experiment in which such Cd concentration had decreased the maize biomass by 50% (Silva *et al.* 2012). Si concentrations of 0.25, 0.5, 1, 1.5 or 2 mmol L^{-1} K_2SiO_3 was also added to the solution and the maize plants were allowed to grow for 21 d. The control was regarded as the treatment without Si but containing 30 µmol(Cd) L^{-1}.

Chl fluorescence measurements: Five ChlF measurements were made throughout the experiment. The first measurement was done before the addition of Cd and the last one a day before the plants were harvested. Measurements were made at night to ensure that electron transport in the photosynthetic apparatus was not functional. Data were obtained from three different positions on two leaves of each plant. The analysis of *in vivo* ChlF was performed using an ultraviolet light emitting diode (LED) as an excitation source. The spectral fluorescence peaks at wavelengths of 685 nm and 735 nm (*USB 2000*, *Ocean Optics,* USA) were analyzed. Spectra were obtained using the software *Ocean Optics-Spectra Suite* (*Ocean Optics*, USA) and fitted with two Gaussian curves corresponding to red (685 nm) and far-red (735 nm) peaks. The F_{685}/F_{735} fluorescence intensity ratios and the peak heights were calculated from the fitted curve for each dose of Si in solution; these data were used to obtain information on the effect of silicon on PSII using the software *Origin 6.0* (*Originlab*, USA).

Chl contents: Leaves were sampled when the plants were harvested to determine the concentrations of Chl *a* and *b*, which together provide the total Chl content (Arnon 1949). Samples were obtained from the middle third of the leaf used for the ChlF analysis. The Chl content was determined in 80% of acetone extract by a spectrophotometer (*NI 2000UV, Nova Instruments*, Brazil), using Arnon (1949) equations for calculations.

Plant biomass and chemical analyses: Leaves, stems, and roots were rinsed once in tap water and three times in distilled water. Subsequently, the samples were kept in a forced air circulation oven at 65ºC until they reached a constant mass. The dry matter of the leaves, stems, and roots was obtained and added together to get the total dry matter.

Digestion of the plant material was done in nitric acid and hydrochloric acid in a microwave oven (*Mars Xpress, CEM Corporation*, USA) according to the 3051A method (USEPA, 1998). The Cd content of the plant material was measured in the digestion extract, using an atomic absorption spectrophotometer (*AAnalyst 800, Perkin Elmer*, USA). The digestion of the plant tissue was done in an autoclave by using hydrogen peroxide and sodium hydroxide. Si was measured by a photocolorimeter at 660 nm (*NI 2000UV, Nova Instruments*, Brazil) using ammonium molybdate as a complexing agent (Korndörfer *et al*. 2004).

Statistical analysis: Each treatment was replicated four times in a randomized block design. The data were then analyzed for variance (*ANOVA*) and regression. The parameters of the fitted regression obtained were tested to significance level of $p<0.05$. All the analyses were carried out with *SPSS 16.0* (*SPSS Inc.*, USA).

Results and discussion

Addition of Si to the nutrient solution did not significantly increase the dry matter production of the plants (Table 1). However, the dry matter production was higher in all plants treated with Si than that in the control receiving only Cd. This trend suggested that in a longer experiment, the effects of Si on alleviating Cd stress could be reflected in increased maize biomass. For instance, Zhang *et al.* (2008) performed a long-term experiment on Cd contamination (105 d) and concluded that a continuous exogenous Si supply alleviated Cd toxicity in rice plants. This was attributed to the increase of biomass and enhanced capacity of the roots to trap Cd through a larger root biomass and hence a higher root Si content. The Si-mediated alleviation of Cd stress may also vary in different plant species and even between plants of the same species (Lukačová Kuliková and Lux 2010). Doncheva *et al.* (2009) reported that Si addition had an amelioration effect on Mn toxicity in a sensitive maize variety Kneja 605, while little effect was observed in the Mn-tolerant Kneja 434.

Table 1. Dry matter production of maize plants under Cd stress and exposed to silicon. ns – not significant. CV – coefficient of variation.

Si [µmol L^{-1}]	Dry matter production [g per pot] Leaf ^{ns}	stem ^{ns}	Root ^{ns}	Total ^{ns}
0.00	12.41	8.50	6.49	28.04
0.25	14.83	10.29	7.48	32.61
0.50	14.96	9.99	7.37	32.32
1.00	14.26	10.25	7.13	31.63
1.50	15.73	10.49	7.38	33.60
2.00	12.80	9.13	7.15	28.45
CV [%]	11.41	13.41	8.16	10.92

Cd phytotoxicity can provoke complex biochemical and physiological alterations. The most well-known symptoms include reduction of plant biomass (Cunha and Nascimento 2009, Feng *et al*. 2010, Marques and Nascimento 2013), imbalance of mineral nutrients (Nascimento *et al*. 1998, Gouia *et al*. 2000) and Chl metabolism (Cheriff *et al*. 2010, 2012; Silva *et al*. 2012). Several authors have shown that Si supplementation can ameliorate some of the toxic effects of Cd in plants. For instance, Si was effective in alleviating Cd toxicity in cucumber plants, resulting in increased dry matter production in the roots (Khadarahmi *et al*. 2012); further, Si also increased the dry matter production of wheat plants grown in soil contaminated with Cd (Rizwan *et al*. 2012).

We noted that the highest Si dose applied (2 mmol L^{-1}) caused a reduction in the dry matter of the plants. This indicates that even though Si is a beneficial element, the application of high doses of Si may cause a nutritional imbalance and reduce the dry matter production of the plants (Araújo *et al*. 2011). The increase in the ratio of Cd either in the leaves or in the stems to that in the roots (Table 2) indicated that Si promoted the reduction of Cd in the aerial plant parts. The 1 mmol L^{-1} dose of Si promoted the highest increase in this ratio. The suppression of Cd uptake and transport is regarded as an efficient defense mechanism against Cd toxic effects (Song *et al*. 2009). Silicon in solution caused an incremental decrease in Cd concentrations in the roots of *Arabidopsis thaliana.* The observed reductions in Cd concentrations in the shoots of this species are due to a reduced translocation of this metal (Cabot *et al*. 2013).

Addition of Si to the nutrient solution increased the Si content of the leaves, stems, and roots (Fig. 1). Accumulation of Si in the shoots confirmed the classification of maize as a Si accumulator (Datnoff *et al*. 2001). In this case, the absorption of Si, most likely, occurred using its active form *via* specific transporters (Lsi1 and Lsi2) (Chen *et al*. 2012). Si taken up by the roots must have moved to the shoots, where it accumulated as a polymer in the leaf apoplast. This forms an important barrier that protects the plant from various types of stress (Mitani *et al*. 2005) or causes coprecipitation of toxic elements in plant tissues (Cunha and Nascimento 2009, Gu *et al*. 2011).

Si reduced the Cd content in the leaves and stems of maize plants (Table 2). The reduction of Cd concentrations in the aerial parts of the plant indicates that Si interfered with the processes of absorption and translocation of Cd to the aerial parts. Inhibition of root-shoot transport of metals

Table 2. Cd concentrations in leaves, stems, and roots of maize plants grown in nutrient solutions with silicon. *,** significant at 5 and 1% probability levels, respectively. ns – not significant. CV – coefficient of variation.

Si [µmol L^{-1}]	Cd concentration [mg kg^{-1}]			Ratio [Cd]	
	Leaf*	Stem*	Root^{ns}	Root/leaf**	Root/Stem**
0.00	137.33	129.83	1,652.42	12.03	12.73
0.25	130.83	106.00	2,040.68	15.60	19.25
0.50	135.12	118.52	1,634.68	12.10	13.79
1.00	94.07	94.67	2,138.19	22.73	22.86
1.50	110.24	94.42	1,632.39	14.81	17.29
2.00	104.08	78.35	1,191.82	11.45	15.21
CV [%]	2.47	14.76	22.19	19.05	21.85

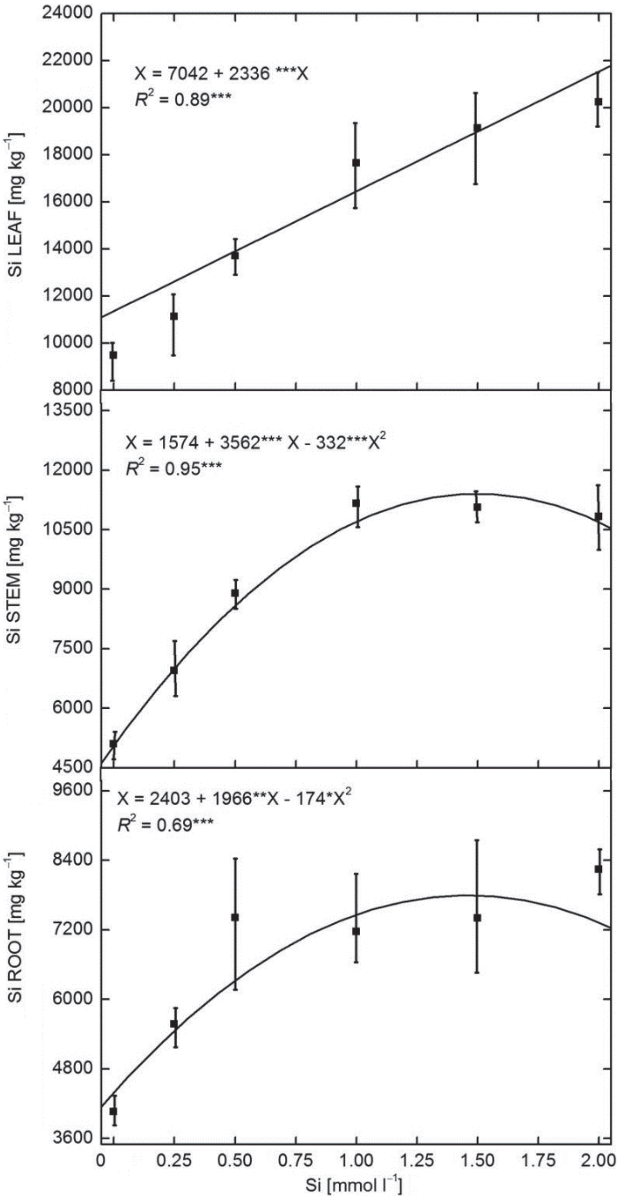

Fig. 1. Silicon concentrations in the leaves, stems, and roots of maize plants grown in a nutrient solution contaminated with Cd with increasing doses of Si. *,**,*** – significant at 5, 1, and 0.1% probability levels, respectively.

is one of the main Si-mediated mechanisms against metal toxicity (Liang *et al.* 2007).

Addition of silicon is known to reduce the toxicity of Cd and significantly decrease the absorption and translocation of this metal from the roots to the aerial parts of *Brassica chinensis* grown in a nutrient solution (Song *et al.* 2009, Liu *et al.* 2013). Silicon reduces the availability of Cd in the soil, making the sequestration of Cd in plant roots more efficient and thus limiting the translocation of this element to the aerial parts of the plant (Rizwan *et al.* 2012). Ye *et al.* (2012) reported that Si increases the proportion of Cd in the apoplast and reduces the amount of Cd in the symplasm. This reinforces the theory that Si enhances the binding of Cd to the cell walls and limits the transport of Cd through the apoplastic pathway. The reduction in the translocation of Cd to the aerial parts of the plant reflects the importance of Si in protecting plant tissue from the various possible effects of Cd.

Concentrations of photosynthetic pigments did not significantly change with the addition of Si to the nutrient solution (Table 3). Although insignificant, a trend towards increasing concentrations of Chl *a* and Chl *b* was observed when 1 or 2 mmol L^{-1} of Si was used. Nwugo and Huerta (2008) have suggested that the increase in PSII performance of Cd-stressed plants after Si treatment is most likely due to Si-induced alleviation of Cd-mediated damage to PSII components and not due to reduced Chl content. The concentrations of photosynthetic pigments do not always change with the application of Si in maize

Table 3. Photosynthetic pigment concentrations as a function of Si doses in maize plants grown in a nutrient solution enriched with cadmium. ns – not significant. CV – coefficient of variation.

Si [µmol L^{-1}]	Chlorophyll concentration [mg g^{-1}]		
	a ^{ns}	*b* ^{ns}	Total ^{ns}
0.00	0.61	0.40	1.01
0.25	0.66	0.33	0.99
0.50	0.52	0.31	0.82
1.00	0.62	0.49	1.11
1.50	0.46	0.39	0.85
2.00	0.71	0.51	1.22
CV [%]	27.65	23.63	19.66

plants (Lukačová *et al.* 2013). An increase in the concentrations of photosynthetic pigments was observed in cucumber plants grown under Cd stress and supplied with Si (Feng *et al.* 2010), although with a lower Cd concentration in the solution than that in our experiment. The concentrations of photosynthetic pigments reflected the Cd content found in the leaves (Tables 2, 3), as these concentrations tended to be higher when the Cd content was lower.

Fluorescence emission spectra were useful in differentiating the effects of different concentrations of Si on the alleviation of Cd toxicity in plants (Fig. 2). Analysis of fluorescence emission spectra, obtained in the presence of different concentrations of Si, showed the role of Si in alleviating Cd stress as well as the importance of this technique for the study of Cd detoxification by Si.

In our experiments, both the normalized spectral emission and the intensity of ChlF were affected by the Si treatment. According to the normalized spectral emission data (Fig. 2*A*), the control (Cd without Si) and the highest doses of Si (1.5 and 2 mmol L^{-1}) demonstrated the sensitivity of ChlF in detecting changes in the PSII under stress of these elements. A high concentration of Si is likely to co-precipitate metals that are essential to photosynthetic efficiency, such as iron and copper (Gu *et al.* 2011). Analysis of Chl *a* fluorescence intensity measurements (Fig. 2*B*) showed that both Cd without Si treatment (control) and Si at the highest concentration (2 mmol L^{-1}) provided higher fluorescence emissions than that of Si at 1 mmol L^{-1}. Therefore, this intermediate Si dose seemed to be the best for maintaining the functionality of the PSII in the Cd-stressed plants.

The red and far-red (Fr/Ffr) values at 685 nm and at 735 nm (Fig. 3) demonstrated that Si-mediated effects on Cd toxicity on PSII were time-dependent, and ChlF can detect alterations in photosynthetic pigments five days after Si application. This confirmed the feasibility of this method for detecting early Cd stress amelioration by Si. The Fr/Ffr ratio obtained from ChlF spectra also indicated that Si doses promoted temporal alterations in the PSII, which were highlighted after 15 d of exposure.

The 1 mmol L^{-1} dose of Si was optimal for the Si-mediated alleviation of Cd stress in plants over time. This corroborates the results obtained in the ChlF intensity measurements (Fig. 2) as well as the reduction of the Cd content in the aerial parts of the plant after the addition of 1 mmol L^{-1} of Si (Table 2). Pietrini *et al.* (2003) and Wang *et al.* (2009) observed a decrease of the efficiency of PSII (measured as F_v/F_m) in response to Cd stress; it indicated that the maximum potential quantum yield of PSII was lowered. Si has been shown to exert a positive effect on growth, photosynthesis, and ChlF parameters of plants under Cd stress. Indeed, the addition of Si increases the quantum yield and the maximum photochemical efficiency of PSII (Feng *et al.* 2010). The ChlF analysis also demonstrated that Si alleviated Cd toxicity in rice plants by reducing Fo and increasing the F_v/F_m ratio and q_P value (Nwugo and Huerta 2008).

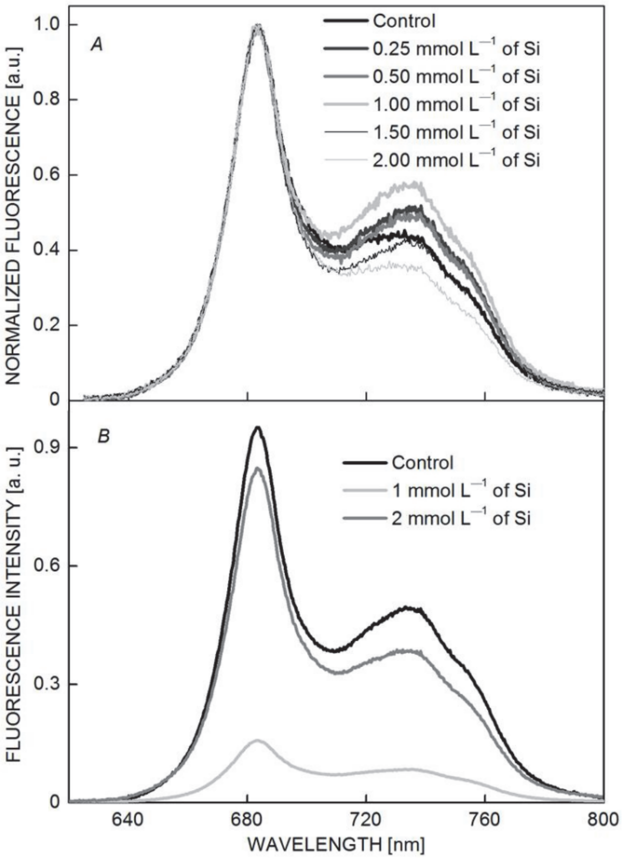

Fig. 2. (*A*) Chlorophyll *a* fluorescence normalized at 685 nm, at room temperature, from maize plants grown under different doses of Si in a nutrient solution contaminated with Cd. (*B*) Intensity of chlorophyll *a* fluorescence for the highest doses of Si (1 and 2 mmol L^{-1}) in the nutrient solution. Control: 30 µmol L^{-1} of Cd without Si.

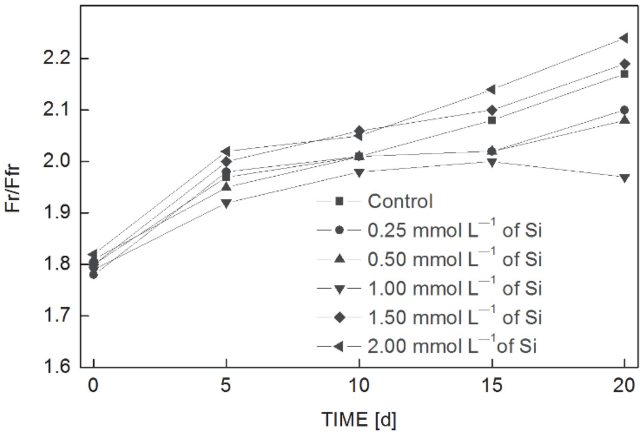

Fig. 3. Ratio of chlorophyll fluorescence spectra as a function of cultivation time of maize plants grown under increasing Si doses in a nutrient solution contaminated with Cd; this ratio refers to the red and far-red (Fr/Ffr) readings at F_{685} and at F_{735}, respectively. Control: 30 µmol L^{-1} of Cd without Si.

Conclusions: Chl fluorescence data and their analysis demonstrated alleviation, by silicon, of Cd toxicity in maize plants grown in a nutrient solution, even when significant changes in dry matter production and concentrations of photosynthetic pigments were not observed. The spectral emission and the Fr/Ffr ratio were sensitive to the effects of Si and represent promising tools for environmental studies. Silicon caused a reduction in the translocation of Cd to the aerial parts of the plant and can be used in green technologies, such as phytoremediation or phytostabilization of contaminated soils.

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