Australian Journal of Crop Science AJCS
AJCS 9(11):1113-1118 (2015) ISSN:1835-2707

Beneficial repercussion of silicon (Si) application on photosynthetic pigments in maize plants

Maria Antonia Machado Barbosa[1], Milton Hélio Lima da Silva[2], Gélia Dinah Monteiro Viana[1], Tiago Rodrigues Ferreira[1], Carla Letícia Figueredo de Carvalho Souza[1], Elaine Maria Silva Guedes Lobato[1], Allan Klynger da Silva Lobato[1,*]

[1]Núcleo de Pesquisa Vegetal Básica e Avanaçada, Universidade Federal Rural da Amazônia, Paragominas, Brazil
[2]Coordenação de Botânica, Museu Paraense Emílio Goeldi, Belém Pará, Brazil

***Correspomnding author: allanllobato@yahoo.com.br**

Abstract

Silicon (Si) is a beneficial element to higher plants. Its effects frequently linked to physiological, morphological, nutritional, and molecular aspects in plants. This study aimed to measure the compounds linked to oxidative stress and cell damages, besides to evaluate the consequences of exogenous Si application on photosynthetic pigments in maize plants (hybrid 30F53YH Pioneer®). The experiment employed an entirely randomized design with two silicon treatments (0 and 2 mM Si). The experiment was assembled with five replicates for a total of 10 experimental units, with one plant in each unit. Parameters measured were hydrogen peroxide, glutathione, electrolyte leakage and malondialdehyde, besides chlorophylls and carotenoids. The silicon application did not cause significant changes in hydrogen peroxide, glutathione, electrolyte leakage and malondialdehyde in leaf and root tissues of plant. On the other hand, the exogenous Si induced significant increases in CHL *a*, CHL *b* and total CHL. This study proved that silicon promotes beneficial effects on photosynthetic pigments and stability of the cell membrane due to maintenance in oxidant compounds in young *Zea mays* plants. Therefore, the application of 2 mM Si can be recommended to maximize the chlorophylls in maize crop, which needs further studies on economic reliability.

Keywords: Chlorophyll; light interception; protection; silicon; *Zea mays*.
Abbreviations: ASC_ascorbate, ATP_adenosine triphosphate, CAR_carotenoids, CHL a_chlorophyll a, CHL b_chlorophyll b, EL_Electrolyte leakage, GR_glutathione reductase, GSH_glutathione, GSSG_glutathione disulphide, H_2O_2_hydrogen peroxide, MDA_malondialdehyde, NADPH_ nicotinamide adenine dinucleotide phosphate, O_2^-_superoxide, ROS_reactive oxygen species, Si_silicon, TOTAL CHL_total chlorophyll.

Introduction

Silicon (Si) is a beneficial element to higher plants (Epstein and Bloom, 2004). Its absorption and deposition in cell walls of several organs such as leaf and stem can promote beneficial effects (Cunha et al., 2008). For this reason it has been frequently linked to physiological, morphological, nutritional, and molecular aspects in plants (Ma, 2004; Epstein and Bloom, 2004; Ma and Yamaji, 2006; Lobato et al., 2009).

In plants, the Si is mainly assimilated by roots but the capacity of plant species and tissues to accumulate this element is variable (Chiba et al., 2009). Several monocots such as *Oryza sativa* and *Triticum aestivum* considered as silicon accumulator, with active absorption by root system. It also presents in leaf tissues, when applied at levels normally higher that 10.0 g kg^{-1} of Si (Oliveira, 2009). On other hand, many dicots like *Phaseolus vulgaris* and *Glycine max* are characterized as silicon non-accumulator. Its passive absorption with leaf tenors begins at 5.0 g kg^{-1} of Si application (Takahashi et al., 1990).

The chlorophylls can be divided in chlorophylls *a* and *b* with greater occurrence into chloroplasts (Candan and Tarhan, 2003). Besides, conversion of radiation to energy in form of ATP and NADPH is largely responsible for interference with photosynthetic structures (Lichtenthaler, 2009). This is directly related to the photosynthetic efficiency in higher plants. In addition, characteristics of these structures are instable and depend on the nutrition supply, sensitivity to light, and denaturation under hot temperatures (Schoefs, 2002).

Reactive oxygen species (ROS) (Asada, 2006), including hydrogen peroxide (H_2O_2) and superoxide (O_2^-) (Queiroz et al., 2002), are highly toxic compounds. ROS promotes the oxidation of membranes and damage essential organelles such as chloroplasts (Carvalho, 2008) and mitochondria (Moller, 2001), which results in cell damage or death (Mittler, 2002). Ascorbate and glutathione (GSH) have essential functions in antioxidant metabolism (Wang et al., 2011) because ascorbate (ASC) is used as a substrate (Mehlhorn et al., 1996). Additionally, GSH produces ascorbate and glutathione disulphide (GSSG), which is used to regenerate GSH via glutathione reductase (GR) (Creissen et al., 1999).

This study aimed to measure compounds linked to oxidative stress and cell damages, such as hydrogen peroxide, glutathione, electrolyte leakage and malondialdehyde, besides to evaluate the consequences on photosynthetic pigments, like as chlorophylls and carotenoids in maize plants exposed to exogenous application of silicon.

Results

Effects of silicon treatment on H_2O_2 and GSH

The silicon application promoted an insignificant decrease in H_2O_2 level in leaf (Fig 1 A). This reduction was approximately 17%, if compared to treatment without silicon. On the other hand, the treatment under Si action also induced an insignificant increase of 11% (Fig 1 B) in H_2O_2 level ino root, compared to control plants. The GSH levels of plants treated with Si presented insignificant increase of 13% in leaf (Fig 1 C), compared to control treatment. In root, the silicon treatment promoted a decrease of 28% (Fig 1 D), being insignificant.

Maintenance in EL and MDA after silicon application

The Si application induced decrease in EL of leaf (Fig 2 A). To EL in root was showed increase of 3% after silicon treatment (Fig 2 B). In addition, the modifications in leaf and root were not significant. The MDA value in leaf tissue presented increase of 3% (Fig 2 C) after Si treatment, compared with control plants. In root, the silicon induced an insignificant decrease of 2% in MDA levels (Fig 2 D).

Beneficial effects produced by silicon in photosynthetic pigments

The Si application promoted significant increase of 22% in CHL *a* level, compared to control treatment (Fig 3 A). The CHL *b* level suffered also significant increase of 43% after silicon treatment (Fig 3 B). In CHL total, this increase was approximately 26% (Fig 3 C) in silicon treated plants. The exogenous silicon occasioned an insignificant decrease in CAR level (Fig 3 D), compared to control treatment. The Si induced insignificant changes in H_2O_2, GSH, EL and MDA levels in both tissues. On the other hand, the exogenous Si produced significant increases in CHL *a*, CHL *b* and total CHL.

Discussion

The silicon application produced a decrease in H_2O_2 level in leaf. This behavior can be associated to characteristic of this mineral to improve the capacity of plant defense against oxidative damages, because it will increase the activity of enzyme antioxidants (Bharwana et al., 2013). According to Sayed and Gadallah (2014), the treatment with 1 mM Si significantly decreased the H_2O_2 level, whereas we observed that H_2O_2 level insignificantly affected, although the concentration of Si was doubled (2 mM Si). Fortunato et al. (2012) evaluated the silicone effect in root of *Musa* sp. also showed increase in H_2O_2 levels, similar to results described in this study.

The increase in Si concentration induced increase in GSH in leaf. This must be related to the fact that GSH is an antioxidant compound with function to protect enzymes and protein structures from sulfhydryl (thiol) group against oxidation Li (Liang et al., 2006). Similar result on increase in GSH was reported by Ali et al. (2013) working on *Helianthus annuus* plants under Si treatment. Wang et al. (2011) studied the Si effects in *Medicago sativa* plants and observed decrease in GSH levels, being corroborated with this study.

The decrease in EL in leaf occasioned by Si treatment suggests that this element contributed in protection of antioxidant system and consequently in attenuation of oxidative stress (Gharrineh and Karmollachaab, 2013). Similar result was found by Nasseri et al. (2012) evaluating *Trigonella foenum-graceum* plants subjected to Si treatment. Zhu et al. (2004) investigating *Cucumis sativus* plants showed increase in values of electrolyte leakage in root under Si application, being corroborated with results of this study.

The increase in Si concentration promoted a slight increase in MDA values. This fact can be attributed to decrease in membrane permeability and consequently small MDA accumulation after Si application (Shahnaz et al., 2011). Similar results were reported by Mohsenzadeh et al. (2011), using Si in *Z. mays* plants. Kim et al. (2014), worked on *Oryza sativa* plants and observed minor values of MDA in root after Si treatment, being also described in this study.

The CHL *a* and CHL *b* levels presented increases in response to Si application. These increases are explained by the fact that Si is absorbed by plant and consequently accumulated in epidermis, which promotes beneficial changes in plant structure and better light capture by the leaf (Locarno et al., 2011). Zago et al. (2010) also observed the increase in *CHL* a in *Glycine max* plants exposed to silicon treatment. Study conducted by Nabati et al. (2013) working on *Sorghum bicolor* plants under Si treatment showed increase in CHL *b*, corroborating with this study.

The Si application produced increase in total CHL due to combined increase in CHL *a* and CHL *b*. The increase in total *CHL* amount normally is associated with improvement in light interception and better performance of the photosynthetic parameters. Xie et al. (2014) evaluated the Si effects in *Z. mays* plants and observed increase in total CHL, similar to our results described in this study. Results described by Curvelo et al. (2013) revealed similar results on decrease promoted by the silicon in *Gossypium hirsutum* plants.

Materials and Methods

Location and growth conditions

The experiment was performed on the Campus of Paragominas of the Universidade Federal Rural da Amazônia, Paragominas, Brazil (2°55'S and 47°34'W). The study was conducted in a greenhouse with environmental control, and the minimum, maximum, and median temperatures were 21°C, 35°C, and 25°C, respectively. The relative humidity during the experimental period varied between 45% and 72%, and the photoperiod was set to 12 h of light. During the measurement period (12:00 h), the amount of photosynthetically active radiation varied between 466 and 1,492μmol m^{-2} s^{-1}.

Plant material, substrate and containers

Seeds of *Zea mays* L. hybrid, 30F53YH Pioneer® were germinated and grew in 1.2-L pots (0.15 m in height and 0.10 m in diameter), filled with the mix substrate composed by sand and vermiculite in 3:1 proportion, respectively. For semi- hydroponic cultivation, pots described previously were equipped with one hole at the bottom side and covered with mesh to keep the substrate, and solution absorption by capillarity, being placed into other containers (0.15 m in height and 0.15 m in diameter) containing 500 mL of distillated water for four days. After that, the nutritive solution of Hoagland and Arnon (2011) was used.

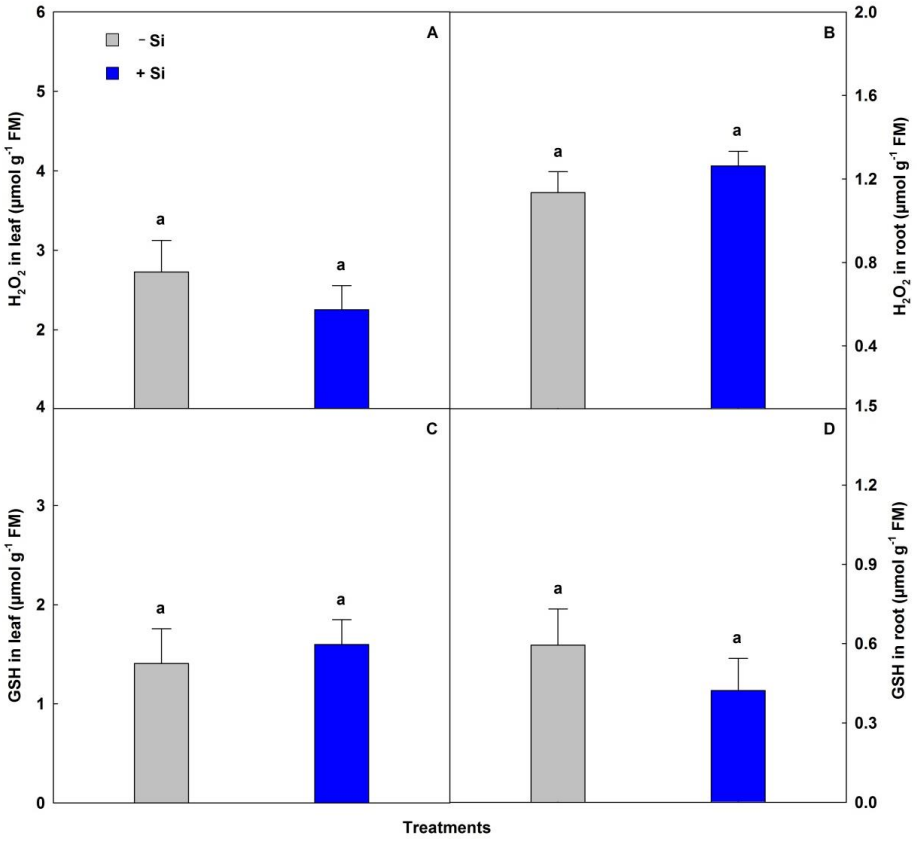

Fig 1. Hydrogen peroxide in leaf and root (A and B), as well as glutathione in leaf and root (C and D) in *Zea mays* plants subjected to silicon treatment. Different letters indicate significant differences at F-test ($P \leq 0.05$). Means ± SD, n = 5.

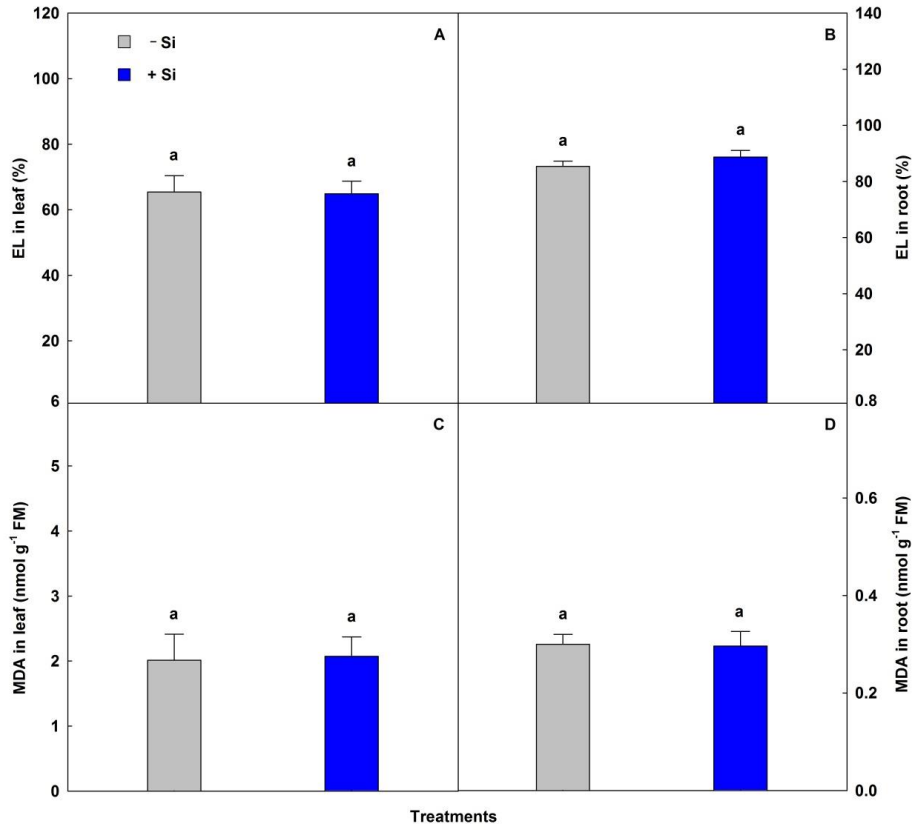

Fig 2. Electrolyte leakage in leaf and root (A and B), as well as malondialdehyde in leaf and root (C and D) in *Zea mays* plants subjected to silicon treatment. Different letters indicate significant differences at F-test ($P \leq 0.05$). Means ± SD, n = 5.

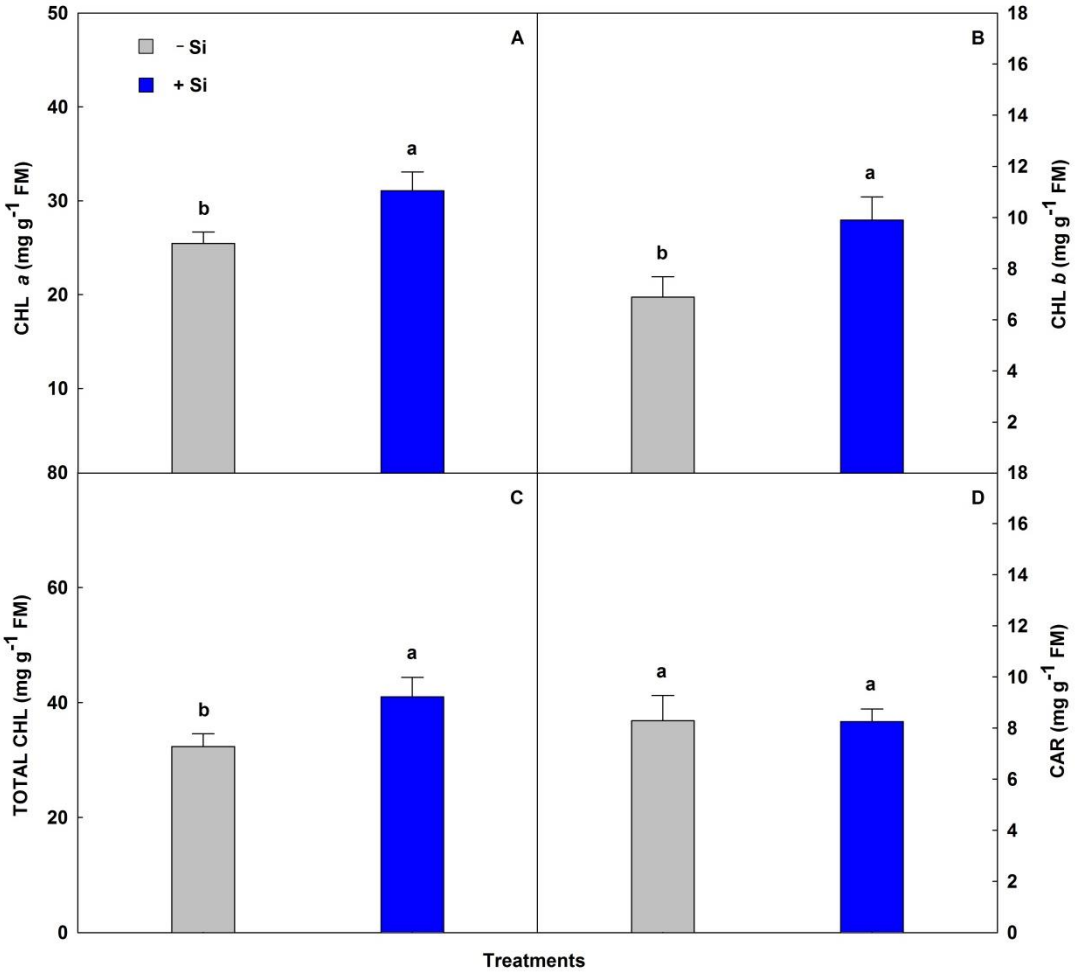

Fig 3. Chlorophyll a (A), chlorophyll b (B), total chlorophyll (C) and carotenoids (D) in Zea mays plants subjected to silicon treatment. Different letters indicate significant differences at F-test ($P \leq 0.05$). Means ± SD, n = 5.

Experimental design

The experiment was setup as an entirely randomized design with two silicon levels (0 and 2 mM Si). The experiment was assembled with five replicates for a total of 10 experimental units, with one plant in each unit.

Plant growing and Si treatment

Five seeds were placed in each pot, and each pot was limited to one seedling in 7th day after seed emergence. All plants during seven days received only distillated water, and after this period nutritive solution, being increased the ionic forces of 25, 50 and 100% at 8th , 9th and 10th day, respectively, after experiment implementation. The treatments received macronutrients and micronutrients from the nutritive solution described by 8.75 mmol KNO_3, 7.5 mmol $Ca(NO_3)_2{\cdot}4H_2O$, 3.25 mmol $NH_4H_2PO_4$, 1.5 mmol $MgSO_4{\cdot}7\ H_2O$, 31.25 µmol KCl, 15.68 µmol H_3BO_3, 1.25 µmol $MnSO_4{\cdot}H_2O$, 1.25 µmol $ZnSO_4{\cdot}7H_2O$, 0.32 µmol $CuSO_4{\cdot}5H_2O$, 0.32 µmol $NaMoO_4{\cdot}5H_2O$, and 200.0 µmol $NaEDTAFe{\cdot}3H_2O$ in a total volume of 0.4 L without silicon addition. To simulate Si action, 2 mM $Na_2SiO_3{\cdot}9H_2O$ was used. All treatments were applied to plants for 20 days. The treatments were changed at 07:00 h over 3-day intervals with their pH adjusted to 5.5 ± 0.1 via the addition of HCl or NaOH. On the 30th day, all plants were physiologically measured and harvested for biochemical evaluations.

Extraction of oxidant and antioxidant compounds

Oxidant and antioxidant compounds (H_2O_2 and GSH) were extracted from leaf and root tissues as described by (Wu et al., 2006). Briefly, an extraction mixture was prepared by homogenising 500 mg of fresh matter in 5 mL of 5% (w/v) trichloroacetic acid. Subsequently, the samples were centrifuged at 15,000 × g for 15 min at 3°C, and the supernatant was collected.

Hydrogen peroxide determination

For H_2O_2 detection, 200 µL of supernatant and 1,800 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).

Glutathione quantification

For GSH detection, 200 µL of supernatant and 1,800 µL of reaction mixture (containing 100 mM phosphate buffer [pH 7.6] and 0.60 mM 2-nitrobenzoic acid) were combined, and the absorbance was measured at 412 nm (Wu et al., 2006).

Electrolyte leakage

Electrolyte leakage was measured according to the method described by Gong et al. (1998) with minor modifications. Fresh tissues (200 mg) were cut into pieces with a length of 1 cm and were placed in containers containing 8 mL of distilled deionised water. The containers were incubated in a water bath at 35°C for 30 min, and the initial electrical conductivity of the medium (EC_1) was measured. The samples were boiled at 95 °C for 20 min to release the electrolytes. After the samples were cooled, the final electrical conductivity (EC_2) was measured (Gong et al., 1998). The percentage of electrolyte leakage was calculated using the formula EL (%) = $EC_1 / EC_2 \times 100$.

Malondialdehyde quantification

MDA was determined by mixing 500 µL of supernatant with 1,000 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 20min, and the reaction was 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 amount of non-specific absorption at 600 nm was subtracted from the absorbance data. The amount of MDA–TBA complex (red pigment) was calculated based on the method of Cakmak and Horst (1991) with minor modifications, using an extinction coefficient of 155 mM^{-1} cm^{-1}.

Determination of chlorophyll content

The determination of the photosynthetic pigments was carried out with 40 mg of leaf tissue. The samples were homogenized in the dark and in the presence of 8 mL of methanol at 90% (Nuclear). Subsequently, the homogenate was centrifuged at 6.000 *g* by 10 minutes in the temperature of 5ºC. The supernatant was removed and the chlorophyll *a* and *b*, carotenoids and total chlorophylls were quantified using spectrophotometer Bel Photonics (UV-M51), according to the methodology of Lichtenthaler and Buschmann (2001).

Data analysis

The data were subjected to an analysis of variance, and significant differences between the means were determined using the F-test at a probability level of 5% (Steel et al., 2006). Standard deviations were calculated for each treatment. The statistical analyses were performed using Assistat software.

Conclusions

The silicon application promoted insignificant changes in H_2O_2, GSH, EL and MDA levels of leaf and root tissues of *Zea mays*. On the other hand, the Si exogenous induced significant increases in CHL *a*, CHL *b* and total CHL. This study proved that silicon promotes beneficial effects on photosynthetic pigments and stability of the cell membrane due to maintenance in oxidant compounds in young *Z. mays* plants. Therefore, the application of 2 mM Si can be recommended to maximize the chlorophylls in maize crop. Further studies are necessary to evaluate the economic importance of Si application.

Acknowledgements

This research financially supported by from Fundação Amazônia Paraense de Amparo à Pesquisa (FAPESPA/Brazil), Conselho Nacional de Desenvolvimento Científico e Tecnólogico (CNPq/Brazil) and Universidade Federal Rural da Amazônia (UFRA/Brazil) to Lobato AKS, as well as Barbosa MAM, Souza CLFC, Ferreira TR and Viana GDM whom were supported by undergraduate scholarships also from Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq/Brazil).

References

Ali MAM, Ramezani A, Far SM, Asilan KS, Ghahderijan MM, Jamian SS (2013) Application of silicon ameliorates salinity stress in sunflower (*Helianthus annuus*L.) plants. Int J Agric Crop Sci. 6:1367-1372.

Asada K (2006) Production and scavenging of reactive oxygen species in chloroplasts and their functions. Plant Physiol. 141:391-396.

Bharwana SA, Ali S, Farooq MA, Iqbal N, Abbas F, Ahmad MSA (2013) Alleviation of lead toxicity by silicon is related to elevated photosynthesis, antioxidant enzymes suppressed lead uptake and oxidative stress in cotton. J Biorem Biodegrad. 4:187.

Cakmak I, Horst WJ (1991) Effect of aluminium on lipid peroxidation, superoxide, dismutase, catalase, and peroxidase activities in root tips of soybean (*Glycine max*). Physiol Plantarum. 83: 463-468.

Candan N, Tarhan L (2003) Relationship among chlorophyll-carotenoid content, antioxidant enzyme activities and lipid peroxidation levels by Mg^{2+} deficiency in the *Menthapulegium*leaves. Plant Physiol Biochem. 41:35-40.

Carvalho MHC (2008) Drought stress and reactive oxygen species. Plant Signal Behav. 3:156-165.

Chiba Y, Mitani N, Yamaji N, Ma JF (2009) HvLsi 1 is a silicon influx transporter in barley. Plant J. 57:810–818.

Creissen G, Firmin J, Fryer M, Kular B, Leyland N, Reynolds H, Pastori G, Wellburn, F, Baker N, Wellburn A, Mullineaux P (1999) Elevated glutathione biosynthetic capacity in the chloroplasts of transgenic tobacco plants paradoxically causes increased oxidative stress. Plant Cell. 11:1277-1291.

Cunha KPV, Nascimento CWA, Accioly AMA, Silva AJ (2008) Cadmium and zinc availability, accumulation and toxicity in maize grown in a contaminated soil. Rev Bras Ciênc Solo. 32:1319-1328.

Curvelo CRS, Rodrigues FA, Pereira LF, Silva LC, DaMatta FM, Berger PG (2013) Leaf gas exchange and oxidative stress on cotton plants supplied with silicon and infected by *Ramularia areola*. Bragantia. 72: 346-359.

Epstein E, Bloom AJ (2004) Mineral nutrition of plants: principles e perspectives. 2nd ed. Sunderland: Sinauer Associates. 400p.

Fortunato AA, Rodrigues FA, Nascimento KJT (2012) Physiological and biochemical aspects of the resistance of banana plants to *Fusarium* wilt potentiated by silicon. Biochem Cell Biol. 102:957- 966.

Gharineh MH, Karmollachaab A (2013) Effect of silicon on physiological characteristics wheat Growth under water-deficit stress induced by PEG. J Agron Plant Prod. 4:1543-1548.

Gong M, Li YJ, Chen SZ (1998) Abscisic acid-induced thermotolerance in maize seedilings is mediated by calcium and associated with antioxidant systems. J Plant Physiol. 153: 488-496.

Hoagland DR, Arnon DI (1950) The water culture method for growing plants without soil.California Agricultural Experiment Station. 1st ed. San Francisco, USA: California Publisher.

Kim Y, Khan AL, Kim D, Lee S, Kim K, Waqas M, Jung H, Shin J, Kim J, Lee I (2014)Silicon mitigates heavy metal stress by regulating P-type heavy metal ATPases, *Oryza sativa* low silicon genes, and endogenous phytohormones. Biomed. Central Plant Biol.14:1-13.

Liang Y, Zhang W, Chen Q, Liu Y, Ding R (2006) Effect of exogenous silicon (Si) on H+-ATPase activity, phospholipids and fluidity of plasma membrane in leaves of salt-stressed barley (*Hordeumvulgare* L.). Environ Exp Bot. 57:212-219.

Lichtenthaler H, Buschmann C (2001) Chlorophylls and carotenoids: Measurement and characterization by UV-VIS Spectroscopy. Curr Protoc Food Anal Chem. F.4.3.1-F.4.3.8, 2001.

Lichtenthaler HK (2009) Biosynthesis and accumulation of isoprenoid carotenoids and chlorophylls and emission of isoprene by leaf chloroplast. Bull Georg Natl Acad Sci. 3:8194.

Lobato AKS, Coimbra GK, Neto MAM, Costa RCL, Santos Filho BG, Oliveira Neto CF, Luz LM, Barreto AGT, Pereira BWF, Alves GAR, Monteiro BS, Marochio CA (2009) Protective action of silicon on relations and photosynthetic pigments in pepper plants induced to water deficit. Res J Biol Sci. 4:617-623.

Locarno M, Fochi C, Paiva PDO (2011) Influence of silicate fertilization on chlorophylls of rose leaves. Ciênc Agropecu. 35: 287-290.

Ma JF (2004) Role of silicon in enhancing the resistance of plants to biotic and abiotic stresses. Soil Sci Plant Nutr. 50:11-18.

Ma JF, Yamaji N (2006) Silicon uptake and accumulation in higher plants. Trends Plant Sci. 11:342-397.

Mehlhorn H, Lelandais M, Korth HG, Foyer CH (1996) Ascorbate is the natural substrate for plant peroxidases. FEBS Letters. 378:203-206.

Mittler R (2002) Oxidative stress, antioxidants and stress tolerance. Trends Plant Sci. 7:405-410.

Mohsenzadeh S, Shahrtash M, Mohabatkar, H (2011) Interactive effects of salicylic acid and silicon on some physiological responses of cadmium-stressed maize seedlings. Iran J Sci Technol. A1:57-60.

Moller IM (2001) Plant mitochondria and oxidative stress: Electron transport, NADPH turnover, and Metabolism of reactive oxygen species. Annu Rev Plant Physiol Plant Mol Biol. 52:561-591.

Nabati J, Kafi M, Massoumi A, Zare M, Mehrjerdi MZ (2013) Effect of salinity and silicon application on photosynthetic characteristics of sorghum (*Sorghum bicolor* L.). Int J Agr Sci. 3: 483-492.

Nasseri M, Arouiee H, Kafi M, Neamati H (2012) Effect of silicon on growth and physiological parameters in fenugreek (*Trigonellafoenum-graceum* L.) under salt Stress. J Agric Crop Sci. 4:1554-1558.

Oliveira LA (2009) Silicon in bean and rice plants: absorption, transport, redistribution, and tolerance to cadmium. [PhD. Thesis] Piracicaba, Escola Superior de Agricultura Luiz Queiroz. 1–157p.

Queiroz CGS, Garcia QS, Lemos Filho JP (2002) Photosynthetic activity and membrane lipid peroxidation in plants mastic-the-wilderness under water stress and after rehydration. Braz J Plant Physiol. 14:59-63.

Sayed SA, Gadallah MAA (2014) Effects of silicon on *Zea mays* plants expoxed to water and oxygen deficiency. Russ J Plant Physiol.61:460-466.

Schoefs B (2002) Chlorophyll and carotenoid analysis in food products. Properties of the pigments and methods of analysis. Trends Food Sci Tech. l3:361-371.

Shahnaz G, Shekoofeh E, Kouroshi D, Moohamadbagher B (2011) Interactive effects of silicon and aluminum on the malondialdehyde (MDA), proline, protein and phenolic compounds in *Boragoofficinalis* L. J Med Plant Res. 5:5818-5827.

Steel RG, Torrie JH, Dickey DA (2006) Principles and procedures of statistics: a biometrical approach. 666 p. Academic Internet Publishers, Moorpark.

Takahashi E, Ma JF, Miyake Y (1990) The possibility of silicon as an essential element for higher plants. J Agric Food Chem. 2:99–122.

Velikova V, Yordanov I, Edreva A (2000) Oxidative stress and some antioxidant system in acid rain treated bean plants: protective role of exogenous polyamines. Plant Sci. 151:59-66.

Wang CQ, Liu TL, Xu HJ (2011) Ascorbate – Glutathione metabolism during PEG-Induced water deficit in *Trifolium repens*. Russ J Plant Physiol. 58:597-602.

Wang X, Wei Z, Liu D, Zhao G (2011) Effects of NaCl and silicon on ctivities of antioxidative enzymes in roots, shoots and leaves of alfalfa. J Biotechnol. 10:545-549.

Wu QS, Xia RX, Zou YN (2006) Reactive oxygen metabolism in mycorrhizal and non-mycorrhizal citrus (*Poncirus trifoliate*) seedlings subjected to water stress. J Plant Physiol. 163: 1101-1110.

Xie Z, Song F, Xu H, Shao H, Song R (2014) Effects of Silicon on Photosynthetic Characteristics of Maize (*Zea mays* L.) on Alluvial Soil. Sci World J. 2014: 1-6.

Zago AS, Silva CAT, Silva TRB, Viecelli CA, Verona E, Nolla A (2010) Effect of Silicon doses on soybean development. Cultiv Saber. 3: 16-22.

Zhu Z, Wei G, Li J, Qian Q, Yu J (2004) Silicon alleviates salt stress and increases antioxidant enzymes activity in leaves of salt-stressed cucumber (*Cucumis sativus* L.) Plant Sci. 167:527–533.