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Protective Action of Silicon on Water Relations and Photosynthetic Pigments in Pepper Plants Induced to Water Deficit

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Abstract: The aim of the study was to investigate the responses promoted by the external application of silicon on water relations and photosynthetic pigments in *Capsicum annuum* L. plants submitted to water deficiency. The experimental design used was entirely randomized, with 5 treatments (stress, 0.25 μM Si, 1.00 μM Si, 1.75 μM Si and control). The stomatal conductance was significantly influenced by the silicon, in which it were showed the values of 3.4, 16.4, 16.1, 13.7 and 24.3 mmol/m^2/sec in stress, 0.25 μM Si, 1.00 μM Si, 1.75 μM Si and control treatments, respectively. The chlorophyll a level in stress, 0.25 μM Si, 1.00 μM Si, 1.75 μM Si and control treatments presented 4.07, 7.29, 8.04, 7.47 and 8.12 mg g^{-1} FM, respectively. The results indicated that the silicon increased the tolerance to water deficit, in which the leaf relative water content, transpiration, stomatal conductance, chlorophylls a and b, as well as carotenoids were maintenanced in higher levels, if compared with stress plants. In addition, the ratio chlorophylls a/b under the 1.00 μM Si was to improve that control treatment.

Key words: *Capsicum annuum* L., silicon, abiotic stress, water restriction, pepper plants

INTRODUCTION

The Silicon (Si) is not considered an essential element for higher plants (Marschner, 1995), despite it to be accumulated in level equivalent to macronutrients as calcium, magnesium and phosphorous (Epstein, 1999). The benefic effects of this element promote to plants the improvement in the tolerance to abiotic stresses as water deficit, salt stress, heavy metal toxicity (Gunes *et al*., 2007) in crops as wheat (Gong *et al*., 2005), sorghum (Hattori *et al*., 2005) and cucumber (Zhu *et al*., 2004).

The water deficiency promotes biochemical changes as accumulation of organic compounds (Costa *et al*., 2008; Lobato *et al*., 2008a), physiological as photosynthesis and stomatal conductance reductions (Ribas-Carbo *et al*., 2005) and morphological as decrease of shoot and increase of the root (Lobato *et al*., 2008b), in which these modifications normality reduces the yield in several crops (Showemimo and Olarewaju, 2007).

The water relations are strongly influenced by the environment, in which the water deficit normality promotes photosynthesis and transpiration decreases (Inamullah and Isoda, 2005), as well as reduction of the stomatal conductance and consequent increase in the stomatal resistance (Ismail *et al*., 2004).

The photosynthetic pigments are formed by the chlorophylls and carotenoids, which it carry out the function of reception and storage light mainly in the antenna complexes (Taiz and Zeiger, 1998). Turan *et al.* (2007) showed reduction in the chlorophyll contents in *Triticum aestivum* plants induced to salt stress.

The aim of the study was to investigate the responses promoted by the external application of silicon on water relations and photosynthetic pigments in *Capsicum annuum* L. plants submitted to water deficiency.

MATERIALS AND METHODS

Growth conditions: The study was carried out in the Instituto de Ciencias Agraria (ICA) of the Universidade Federal Rural da Amazonia (UFRA), Belem city, Para state, Northern region, Brasil (01°27′S and 48°26′W) in the period of February and April of 2008. The plants remained in glasshouse environment under natural conditions day/night (air temperature minimum/maximum and

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relative humidity of 22.1/35.5 °C and 65/93 %, respectively. The photoperiod medium was of 12 h of light and photosynthesis radiation active maximum of 720 μmol/m^2/sec (at 12:00 h).

Plant materials: *Capsicum annuum* L., seeds of the Vermelho Gigante cultivar used in this study were harvested in the 2007 season and coming from Universidade Federal Rural da Amazonia (UFRA), estado Para, Brasil. The seed treatment was carried out through of immersion in solution of N-(triclorometil)-4 ciclohexan 1, 2 dicarbomixid ($C_9H_8Cl_3NO_2S$) at 3 ppm by 30 sec and drying in oven with forced air circulation at 30°C by 120 h (Machado, 2000). The seeds were kept in bags hermetically closed, in which it were remained in the dark and under the temperature of 10°C.

Substrate and pot: The substrate used to the plant growth was composed by sand and silic in the proportion of 2:1, respectively, as well as this substrate was autoclaved at 120°C atm^{-1} by 40 min. The container used to the plant growing was Leonard pot with 2 L capacity and it was adapted in the Laboratorio de Fisiologia Vegetal Avancada (LFVA).

Experimental design and treatments: The experimental design used was entirely randomized, with 5 treatments (stress, 0.25 μM Si, 1.00 μM Si, 1.75 μM Si and control). The experiment was composed by 5 repetitions and 25 experimental units, as well as 1 plant in each unit.

Plant conduction and silicon application: Five seeds pot^{-1} were sowed and after the germination were kept 1 plant pot^{-1}. The stress and control treatments received macro and micro nutrients in the form of nutritive solution of Schwarz (1995), without Silicon (Si). Whereas, the treatments under 0.25 μM Si, 1.00 μM Si and 1.75 μM Si received macro and micro nutrients in the form of nutritive solution previously cited, with addition of silicon through sodium metasilicate ($Na_2SiO_39H_2O$), in agreement with Liang *et al.* (2006) and adapted in Laboratorio de Fisiologia Avancada (LFVA). The solutions were applied in plants by the period of 65 days, as well as the nutritive solution were changes with 5 days of interval, always at 09:00 h and the pH of the nutritive solution was adjusted to 6.0±0.1 with addition of HCl or NaOH. In the 65th day after the experiment implementation, the plants of the treatments under stress, 0.25 μM Si, 1.00 μM Si and 1.75 μM Si were submitted to period of 6 days without nutritive solution, in which the water deficit was simulated in the 65th until 71th day after of the experiment start. After this period, plants were physiologically analysed.

Leaf relative water content: The leaf relative water content was evaluated with leaf disks with 10 mm of diameter and it was carried out in each plant, in which were removed 40 disks and the calculation in agreement with the formula proposed by Slavick (1979):

$$\text{LRWC} = \frac{(\text{MF-MS})}{(\text{MT-MS})} \times 100$$

where:

MF = Matter fresh

MT = Matter turgid evaluated after 24 h and saturation in deionized water at 4°C in dark

MS = The dry matter determined after 48 h in oven with forced air circulation at 80°C

Leaf gas exchange: The leaf temperature, transpiration rate and stomatal conductance were evaluated in leaves totality expanded, under light and present in the medium 3rd of the branch main, in which it was used porometer of state static (LICOR AM-300, model 1600), with the gas exchanges evaluated immediately during the period between 10:00 and 12:00 h in all the plants of the experiment.

Chlorophyll contents: The determination of the photosynthetic pigments was carried out with 25 mg of leaf tissue removed from the 1st leaf, where, the samples were homogenized in the dark and in the presence of 2 mL of acetone at 80% (Nuclear). Subsequently, the homogenized samples were centrifuged at 5.000 g, for 10 min at 5°C. The supernatant was then removed and chlorophyll a, b and carotenoids were quantified using spectrophotometer Femto (700 S). This is in agreement with the methodology of Lichthenthaler (1987).

Data analysis: The data were submitted at variance analysis and when significant differences occurred were applied to Scott-Knott test at 5% level of error probability, as well as the standard errors were calculated in all evaluated treatments (Gomes, 2000). The statistical analysis were carried out with the software SAS Institute (1996).

RESULTS

Effects of Si on leaf relative water content: The leaf relative water content was significantly modified in this study (Fig. 1a), as well as stress, 0.25 μM Si, 1.00 μM Si, 1.75 μM Si and control treatments presented 51.9, 66.1, 68.9, 74.4 and 92.6%, respectively. The values obtained revealed that the increase in the silicon concentration promoted the increase of the water retention in the leaf tissue.

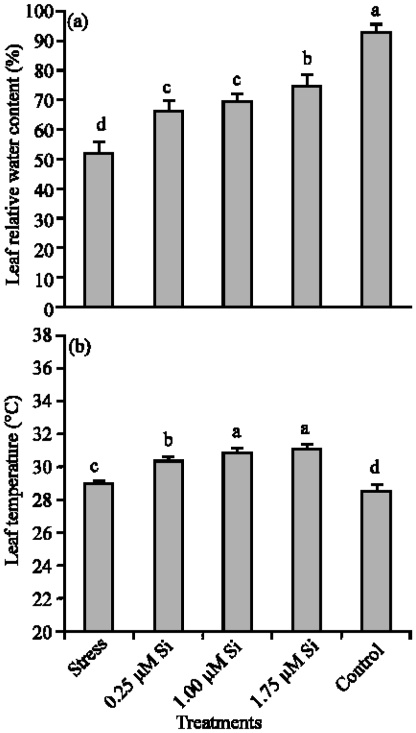

Fig. 1: Leaf relative water content, a): Leaf water content, b): In *Capsicum annuum* cv. Vermelho Gigante under the treatments control, 0.25 µM Si, 1.00 µM Si, 1.75 µM Si and stress. Averages followed by the same letter do not differ among themselves by the Scott-Knott test at 5% of probability. The bars represent the mean standard errors

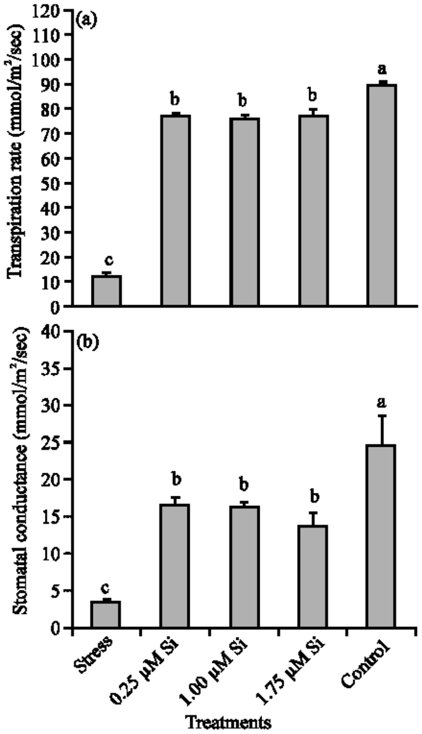

Fig. 2: Transpiration rate, a): Stomatal conductance, b): *Capsicum annuum* cv. Vermelho Gigante under the treatments control, 0.25 µM Si, 1.00 µM Si, 1.75 µM Si and stress. Averages followed by the same letter do not differ among themselves by the Scott-Knott test at 5% of probability. The bars represent the mean standard errors

Effects of Si in leaf temperature: The leaf temperature suffered significant influence, which the stress, 0.25 µM Si, 1.00 µM Si, 1.75 µM Si and control treatments presented the temperatures of 28.9, 30.4, 30.9, 31.1 and 28.5°C, respectively (Fig. 1b). The results prove that the silicon application promoted increase of the leaf temperature.

Changes promoted by Si in transpiration: The transpiration rate was changed after the application of the treatments, in which it was showed the rates of 0.02, 76.7, 75.9, 76.7 and 88.8 mmol/m^2/sec in stress, 0.25 µM Si, 1.00 µM Si, 1.75 µM Si and control treatments, respectively (Fig. 2a). The results reveal that statistically the differents silicon concentrations promoted similar effects under this species.

Behaviour of the stomatal conductance in Si presence: The stomatal conductance in *Capsicum annuum* plants were significantly influenced by the silicon (Fig. 2b), in which it were showed the values of 3.4, 16.4, 16.1, 13.7 and 24.3 mmol/m^2/sec in stress, 0.25 µM Si, 1.00 µM Si, 1.75 µM Si and control treatments, respectively. Despite the stomatal conductance to present decrease under the concentrations of 0.25 µM Si, 1.00 µM Si and 1.75 µM Si, when compared with control plants, the reduction was not significant among these treatments.

Effects of Si in chlorophyll a: The chlorophyll a level suffered action of the silicon application, as well as in stress, 0.25 µM Si, 1.00 µM Si, 1.75 µM Si and control treatments presented 4.07, 7.29, 8.04, 7.47 and 8.12 mg g^{-1} FM, respectively (Fig. 3a). The control and 1.00 µM Si were significantly similars and higher that others treatments. In addition, the treatments under concentrations of 0.25 and 1.75 µM Si were statistically similars.

Effects of Si in chlorophyll b: The results obtained on chlorophyll b level prove the silicon action in the species under study, in which were showed 2.58, 4.43, 4.25, 4.20 and 4.65 mg g^{-1} FM in stress, 0.25 µM Si, 1.00 µM Si,

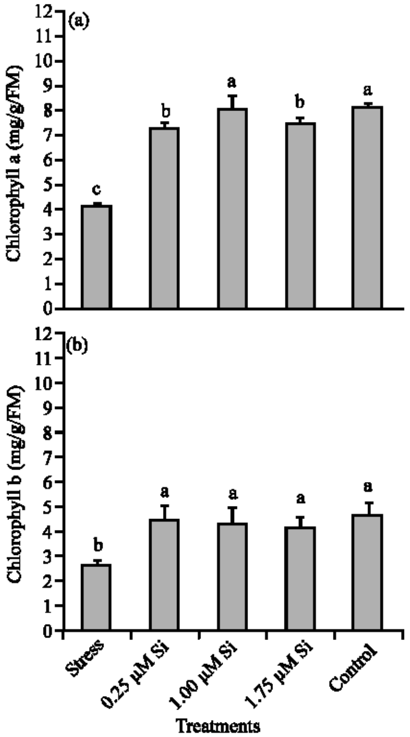

Fig. 3: Chlorophyll a, a): and Chlorophyll b, b): in *Capsicum annuum* cv. Vermelho Gigante under the treatments control, 0.25 µM Si, 1.00 µM Si, 1.75 µM Si and stress. Averages followed by the same letter do not differ among themselves by the Scott-Knott test at 5% of probability. The bars represent the mean standard errors

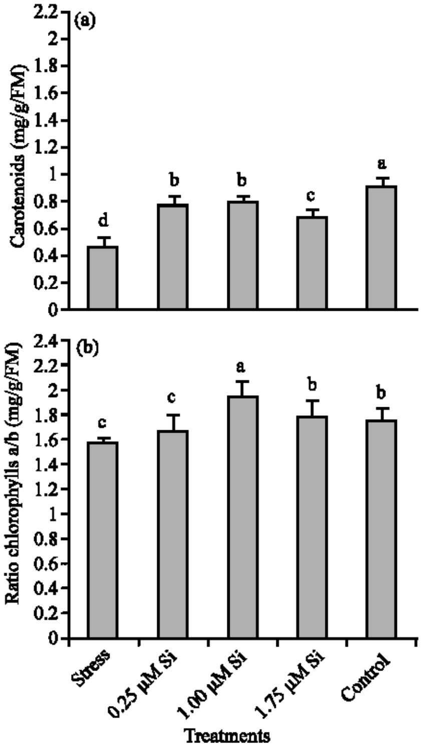

Fig. 4: Carotenoids, a): Ratio chlorophylls a/b, b): In *Capsicum annuum* cv. Vermelho Gigante under the treatments control, 0.25 µM Si, 1.00 µM Si, 1.75 µM Si and stress. Averages followed by the same letter do not differ among themselves by the Scott-Knott test at 5% of probability. The bars represent the mean standard errors

1.75 µM Si and control treatments, respectively (Fig. 3b). The experiment revealed that the concentrations of 0.25 µM Si, 1.00 µM Si and 1.75 µM Si are significantly similars, as well as the exogenous silicon kept this pigment statistically equal to control.

Consequences of Si in carotenoids: The carotenoids presented significant differences, in agreement with variance analysis, as well as concentrations of 0.25 µM Si and 1.00 µM Si were statistically similars and differents of the others treatments. The carotenoids amounts showed were 0.45, 0.76, 0.78, 0.68 and 0.90 mg g^{-1} FM in the stress, 0.25 µM Si, 1.00 µM Si, 1.75 µM Si and control treatments, respectively (Fig. 4a).

Modifications in the ratio chlorophylls a/b promoted by Si: The ratio chlorophylls a/b proves the influence of the silicon application in these pigments, in which the treatment with 1.00 µM Si had higher value and statistically different of all the treatments (Fig. 4b). In addition, the ratio presented in the stress, 0.25 µM Si, 1.00 µM Si, 1.75 µM Si and control treatments the values of 1.57, 1.67, 1.94, 1.78 and 1.75 mg g^{-1} FM, respectively.

DISCUSSION

The leaf relative water content of plants submitted to external silicon were kept in higher levels that the plants under stress, as well as this study reveals the benefic effects of silicon and proves that the stress applied was sufficient to produce significant changes in stress treatment. The leaf relative water content is normality used to evaluate the water volume/amount presents in plant tissue, besides to indicate easily when the plants are under water deficiency due the lower assimilation or total restriction of the water presents into substrate (Lobato *et al*., 2008b; Pimentel, 2004). Study conducted by Degl'Innocenti *et al.* (2008) investigating the dehydration and rehydration effects in *Ramonda serbica* corroborate the results found on leaf relative water.

The increase in the leaf temperature of the plants treated with silicon occurred probability due the silicon deposition in cell walls (Ma and Takahashi, 2002). This parameter suffers direct influence of the water, dry matter and nutrients present in organ, which it is an important indicator on work of the cold system, with the objective to avoid heating and consequent denaturation of membranes, cell compounds, primary and secondary metabolics (Taiz and Zeiger, 1998). Results obtained by Henriet *et al.* (2006) working with *Musa* sp. revealed that old leaves accumulate higher silicon amount that young leaves.

The transpiration maintenance of the plants submitted to exogenous silicon occurred due the leaf relative water content to remain high in these plants, which the water keep turgid stomatal and make possible the transpiration rate in higher levels that the treatment under stress. The transpiration can occur through of 2 forms, in which the primary is the stomatal normality presents in leaves and the secondary is the cuticle presents normality in stems (Kerbauy, 2004). Similar results, on the transpiration maintenance were found by Romero-Aranda *et al.* (2006) investigating the silicon application in *Lycopersicon esculentum* plants submitted to salt stress.

The decrease showed in the stomatal conductance of the plants treated with silicon, in agreement with the increase of the silicon concentration, it was promoted by the accumulation of this element in parts of the leaves, which probably it was formed structures denominated phytoliths in the upper epidermis, as well as parts of palisade mesophyll and lower epidermis around the stomatal including guard cells (Morakawa and Saigusa, 2004). Gunes *et al.* (2007) obtained similar results when evaluated the silicon effects in *Spinacia oleracea* plants under boron toxicity. Results showed by Liu *et al.* (2005) indicate that the water deficit promotes strong decrease in stomatal conductance.

The chlorophyll a level was maintained in the treatment that was applied 1.00 μM Si, in relation to control plants, it reveals that the concentrations of 0.25 μM Si and 1.75 μM Si promote increases, when compared with stress plants; however these concentrations can be considered as sub and supra optimum, because its provoke reduction of chlorophyll a, if compared with control treatment. In agreement with Liang *et al.* (1996) the silicon decrease the permeability of the plasma membrane of leaf cells, as well as this element improves the ultra structure of the chloroplasts (Liang, 1998). The chlorophyll a is considered as the main photosynthetic pigment in higher plants, in which this pigment is responsible by the light absorption that promotes the start of the photosynthesis process (Taiz and Zeiger, 1998). Husaini and Abdin (2008) studying the salt stress effects in transgenic and wild plants found that wild plants present reduction in chlorophyll content.

The maintenance of the chlorophyll b of the plants under stress+silicon action in same level of the control treatment was probably promoted by the increase of antioxidant enzyme activities as superoxide dismutase and catalase (Gong *et al.*, 2005). These enzymes had the function to avoid the oxidative stress provoked during situations of abiotic and biotic stresses (Fadzilla *et al.*, 1997; Liang *et al.*, 2003).

The silicon in the amounts of 0.25 μM Si and 1.00 μM Si promoted attenuation of the effects provoked by the water deficit in carotenoids, as well as it reveals that the stress applied was damaging to this pigment. The carotenoids present functions as light receptor and photo-system protector against reactive oxygen species (Ledford and Niyogi, 2005), which it has the capacity to accumulateβ-carotene and xanthophylls (Telfer, 2002). Lobato *et al.* (2009) working with *Phaseolus vulgaris* plants infected by *Colletotrichum lindemuthianum* pathogen revealed that carotenoids are affects by the stress biotic simulated.

The maximization in ratio chlorophylls a/b of the plants submitted to silicon is directly linked with the benefits showed in chlorophylls a and b, in which this ratio indicates that the light absorption was increased and consequently the photo-receptor efficiency. The results obtained in this study promoted by silicon are corroborated by the experiment conducted by Ranganathan *et al.* (2006).

CONCLUSION

The results obtained on water relations and pigments indicated that the silicon increased the tolerance to water deficit, in which the leaf relative water content, transpiration, stomatal conductance, chlorophylls a and b, as well as carotenoids were maintenanced in higher levels, if compared with stress plants. In addition, the ratio chlorophylls a/b under the 1.00 μM Si was to improve that control treatment. This way, this study corroborates the benefic effects of the silicon exogenous application in pepper plants under influences of the water restriction.

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