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Silicon Exercises Influence on Nitrogen Components in Pepper Subjected to Water Deficit?

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Abstract: The aim of this study was to investigate responses promoted by external application of silicon on nitrogen components in *Capsicum annuum* L. plants submitted to water deficiency. The experimental design was completely randomized, with 4 treatments (0, 0.25, 1.00 and 1.75 μM Si). Leaf relative water content suffered significant changes, with values of 51.9, 66.1, 68.9 and 74.4% in 0, 0.25, 1.00 and 1.75 μM Si treatments, respectively. In total soluble proteins under concentrations of 0, 0.25, 1.00 and 1.75 μM Si were obtained values of 3.8, 4.8, 5.5 and 4.9 mg g DM^{-1}, respectively. This study suggests that exogenous silicon alleviates negative effects promoted by water deficiency on leaf relative water content, stomatal conductance, total soluble proteins, total soluble amino acids and glycinebetaine. Furthermore, the nitrate reductase activity were observed significant benefits only at the concentration of 0.25 μM Si+, however in free ammonium was no obtained improvement after silicon application. Proline level was maximized at concentrations of 1.00 and 1.75 μM Si.

Key words: *Capsicum annuum* L., silicon, water relations, nitrogen, drought, Brazil

INTRODUCTION

Silicon (Si) is a common element in the earth's surface and the form present in soil normally available to plants is monosilic acid $(SiOH)_4$ (Epstein and Bloom, 2005). Despite silicon not being considered an essential element for higher plants (Marschner, 1995), it should be accumulated in levels equivalent to macronutrients like calcium, magnesium and phosphorous (Epstein, 1999).

Benefic effects of silicon in plants are linked to action way, where after being absorbed by the root, this element is frequently translocated and deposited in cell walls, interacting with pectins and polyphenols (Currie and Perry, 2007). This results in cell wall fortification and consequent higher plant protection against abiotic and biotic stresses (Pilon-Smits *et al*., 2009). So, this element improves the plant tolerance to abiotic stresses like water deficit, salt stress and mineral stress (Gunes *et al*., 2007a) in crops such as pepper (Lobato *et al*., 2009a), tomato (Romero-Aranda *et al*., 2006), wheat (Gong *et al*., 2005), sorghum (Hattori *et al*., 2005) and cucumber (Zhu *et al*., 2004).

Water deficiency promotes biochemical changes like the accumulation of organic compounds (Costa *et al*., 2008; Lobato *et al*., 2008a), physiological like the decrease in photosynthetic rates and stomatal conductance reduction (Ribas-Carbo *et al*., 2005), besides morphological, such as shoot decrease and root increase (Lobato *et al*., 2008b), where these modifications normally reduce the yield in several crops (Showemimo and Olarewaju, 2007).

Nutritional supplement is fundamental to keep plant biochemical balance and Nitrogen (N) is normally the mineral element most required by the plants (Maman *et al*., 1999). Nitrogenous forms that can be absorbed by the roots are ammonium (NH_4^+) and Nitrate (NO_3^-) (Marschner, 1995), these also make the production of essential metabolic possible, such as nucleotides, enzymes, amino acids and proteins (Nelson and Cox, 2004).

The aim of this study was to investigate the responses promoted by the external application of silicon on nitrogen components in *Capsicum annuum* L. plants submitted to water deficiency.

MATERIALS AND METHODS

Plant material: *Capsicum annuum* seeds from Vermelho gigante cultivar used in this study came from fruits harvested in the 2007 season, during previous field

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experiments implemented under adequate nutritional and edafoclimatic conditions at the Universidade Federal Rural da Amazonia (UFRA), Para State, Brazil (01°27'S and 48°26'W). Seed treatment was carried out through by immersion in a solution of N-(triclorometil)-4 ciclohexan-1, 2 dicarbomixid ($C_9H_8Cl_3NO_2S$) at 3 ppm for 30 sec and drying in an oven with forced air circulation at 30°C for 120 h (Machado, 2000). Harvested and treated seeds, as previously described, were kept in hermetically closed bags, in the dark and at 10°C, in the seed bank of the Universidade Federal Rural da Amazonia (UFRA) under lot identification number (UFRA/2007-512), until the execution of this experiment.

Growth conditions: Study was undertaken at the Instituto de Ciencias Agraria (ICA) of the Universidade Federal Rural da Amazonia (UFRA), Belem City, Para State, Brazil (01°27'S and 48°26'W), from February to April 2008. Plants were grown under glasshouse environment, with natural minimum/maximum day/night, air temperature and relative humidity conditions of 22.1/35.5°C and 65/93%, respectively. The mean photoperiod was of 12 h under light with Photosynthetic Active Radiation (PAR) of 720 μmol/m^2/sec, measured midday at plant level, using a steady-state porometer (LICOR AM-300, model 1600).

Substrate and pot: Substrate used for plant and development growth was composed of sand and silica gel, since silica gel is a stable and chemically inert compound, in the proportion of 2:1 (v:v), respectively. This mixture was autoclaved (120°C atm^{-1} for 40 min) and distributed over Leonard-type pots, where each pot received 2 L of mixture. The experiment was carried out in the Laboratorio de Fisiologia Vegetal Avancada (LFVA).

Experimental design and treatments: The experimental design used was entirely randomized, with 4 treatments (0, 0.25, 1.00 and 1.75 μM Si). This experiment was composed by 6 replicates and 24 experimental units, with each plant being considered as one experimental unit.

Plant conduction, silicon application and evaluation period: Five seeds were placed per each pot and after the emergence, at 10 days, only one plant pot^{-1} was kept. All plants were watered, as well as received macro and micro nutrients by a nutritive solution described by Schwarz (1995), where 400 mL of this solution was added into each pot. Solution changes were performed with 5 days intervals, always at 09:00 h and the pH was adjusted to 6.0±0.1 by adding HCl or NaOH.

The treatment under 0 μM Si (control) was watered only with the nutritive solution previously reported, therefore, without silicon (Si). Whereas treatments (0.25, 1.00 and 1.75 μM Si) received nutritive solution and addition of silicon through sodium metasilicate ($Na_2SiO_3 9H_2O$), in agreement with Liang *et al.* (2006). The 65th day after experiment implementation was characterized by the vegetative stage end for the cultivar used in this study, since the plants of all treatments were exposed to water deficiency, where they were submitted to a period of 6 days under total absence of nutritive solution. This water deficiency was simulated from the 65th until the 71st day after the beginning of the experiment. All plants on the 71st day were physiologically evaluated and harvested for subsequent biochemical analysis.

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

$$LRWC = \left[\frac{(FM-DM)}{(TM-DM)}\right] \times 100$$

Where:

FM = Fresh Matter

TM = Turgid Matter evaluated after 24 h and saturated in deionized water at 4°C in the dark

DM = Dry Matter determined after 48 h in an oven with forced air circulation at 80°C

Stomatal conductance: To determine stomatal conductance was measured under light from full expanded leaves located in branch middle third, using a steady-state porometer (LICOR AM-300, model 1600). The gas exchanges were evaluated immediately during a period between 10:00 and 12:00 h in all experiment plants.

Nitrate reductase activity: Nitrate reductase enzyme extraction (E.C. 1.6.6.1) was carried out with leaf disks until reaching the weight of 200 mg, where the samples were then incubated in 5 mL of extraction buffer (KH_2PO_4 at 0.1 M, KNO_3 at 50 mM, isopropanol at 1% (v v^{-1}) and pH 7.5) for 30 min at 30°C and all the procedures performed in the dark. Enzyme activity quantification was carried out using the method by Hageman and Hucklesby (1971) with the absorbance at 540 nm, using a spectrophotometer (Quimis, model Q798DP).

Leaf dehydration: Leaves were harvested and placed in an oven with forced air circulation at 70°C for 96 h. After this period, leaf dry matter was triturated and the powder was kept in glass containers. The containers remained in the dark at 15°C, until the carrying out the biochemical analysis.

Free ammonium, amino acids and proline: Free ammonium, amino acids and proline were determined with 50 mg of leaf dry matter powder, which was incubated with 5 mL of sterile distilled water at 100°C for 30 min. After homogenized, the mixture was centrifuged to 2.000 g for 5 min at 20°C and the supernatant was removed. Free ammonium quantification was carried out at 625 nm in agreement with Weatherburn (1967), as well as using $(NH_4)_2SO_4$ (Sigma Chemical) as a standard. Total soluble amino acids quantifications were performed at 570 nm according to Peoples *et al.* (1989), utilizing L-asparagine + L-glutamine (Sigma Chemicals) as a standard. Proline quantification was carried out at 520 nm according to Bates *et al.* (1973), employing L-proline (Sigma Chemicals) as a standard.

Total soluble proteins: Total soluble proteins determination was carried out with 100 mg of powder. The mixture was kept in agitation for 2 h and then centrifuged to 2000 g for 10 min at 20°C, subsequently the supernatant was removed. Total soluble protein quantifications were carried out at 595 nm in agreement with Bradford (1976), as well as using albumin bovine (Sigma Chemicals) as a standard.

Glycinebetaine: Glycinebetaine was determined with 25 mg of leaf dry matter powder, where it was incubated with 2 mL of sterile distilled water at 25°C for 4 h, under agitation. After homogenized, it was centrifuged to 10.000 g for 10 min at 25°C and the supernatant removed. Glycinebetaine quantification was carried out at 365 nm according to Grieve and Grattan (1983), using glycinebetaine (Sigma Chemicals) as a standard.

Data analysis: Data were submitted to variance analyses and when significant differences occurred, the to Scott-Knott test at 0.05 of error probability was applied in addition to standard errors being calculated in all evaluated treatments (Gomes, 2000). Statistical analyses were carried out with the software SAS.

RESULTS AND DISCUSSION

Si effects on leaf relative water content and stomatal conductance: Leaf relative water content suffered significant changes (Fig. 1a), where we obtained values of 51.9, 66.1, 68.9 and 74.4% in treatments described as 0, 0.25, 1.00 and 1.75 μM Si, respectively. These results revealed that a high silicon rate promoted better results in this parameter, consequently increasing water retention in leaf tissue.

This parameter in plants exposed to external silicon maintained at higher levels than plants under 0 μM Si (control), as well as this study reveals silicon benefic

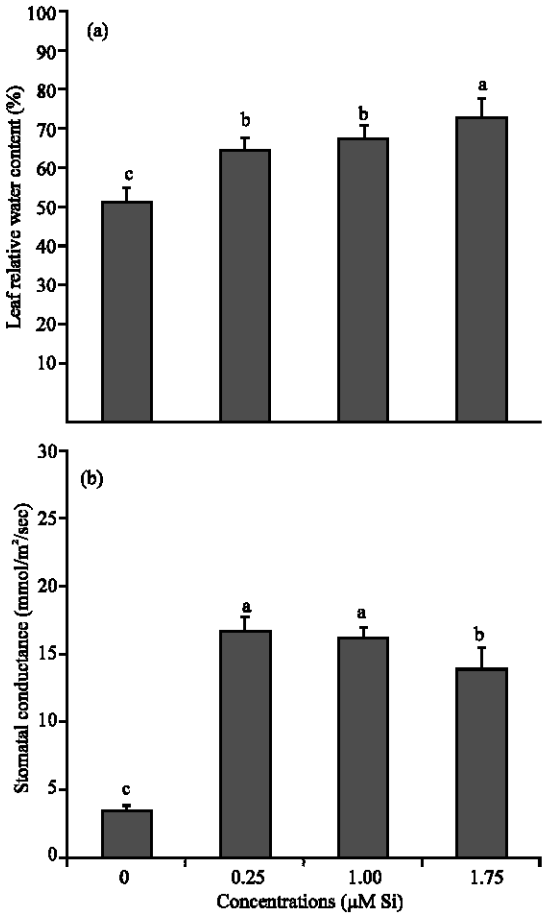

Fig. 1: (a) Leaf relative water content and (b) stomatal conductance in *Capsicum annuum* cv. Vermelho gigante under 4 silicon concentrations (0, 0.25, 1.00 and 1.75 μM Si) and subjected to water deficit. Averages followed by the same letter do not differ among themselves by the Scott-Knott test at 0.05 of probability. The bars represent mean standard errors

effects. This physiological parameter is normally used to evaluate the water volume/amount present in leaf tissue, besides indicating when plants are under a water deficiency situation, due to lower water assimilation or total water restriction (Lobato *et al.*, 2008b; Pimentel, 2004). Studies conducted by Henriet *et al.* (2006) revealed that silicon was found in higher amounts in old leaves than in young leaves, in which these responses were explained based on leaf exposure time.

Results on stomatal conductance were 3.4, 16.4, 16.1 and 13.7 mmol/m^2/sec in 0, 0.25, 1.00 and 1.75 μM Si treatments, respectively (Fig. 1b). So, this study indicates that exogenous silicon significantly affects and minimizes negative effects induced by water deficit on stomatal conductance.

During gas exchanges, strong stomatal closing was obtained in the control treatment (0 μM Si). In addition, the applied silicon was probably deposited in cell walls (Ma and Takahashi, 2002) promoting an increase in stomatal resistance. Therefore, these results suggest that silicon alleviates the negative effects on plants exposed to stress. This parameter normally is affected during water deficiency conditions, with increases in stomatal resistance and consequent reductions in stomatal conductance and transpiration (Lobato *et al*., 2009a), that is a negative consequence to plant. Similar results on decreases in stomatal conductance were obtained by Nogueira *et al*. (1998), working with two *Arachis hypogaea* L. cultivars induced to water deficit, as well as by Figueiredo *et al*. (1999) studying *Vigna unguiculata* (L.) Walp.

Si responses on nitrate reductase activity and free ammonium: Nitrate reductase activity was significantly modified, as well as higher activity being observed in 0.25 μM Si concentration (Fig. 2a). The values obtained in this study were 0.36, 0.67, 0.42 and 0.48 μmol NO_2^-/g/h in 0, 0.25, 1.00 μM and 1.75 μM Si, respectively. Enzyme activities in treatments on silicon action were kept above control treatment.

Enzyme activities in control (0 μM Si), 1.00 and 1.75 μM Si treatments were less active than the 0.25 μM Si treatment. Silicon did not present direct influences on enzyme activity; however the activation form is indirect and probably linked to benefits obtained in protein levels, because the mRNA synthesis of nitrate reductase enzyme is regulated by proteins (Ferrario-Mery *et al*., 1998). This enzyme is considered by Lobato *et al*. (2008c) as an excellent physiological indicator and it can be used in plants submitted to water stress, since nitrate reductase presents advantages by being the first enzyme in the nitrogen metabolism, highly sensitive and consequently, having a quicker response. The results obtained on reduction in nitrate reductase activity are corroborated by Silveira *et al*. (2001) investigating water deficiency in *Vigna unguiculata* plants.

In free ammonium the exogenous silicon promoting a non-significant increase in free ammonium (Fig. 2b) and treatments under 0.25, 1.00 and 1.75 μM Si presented the values of 20.5, 20.3 and 22.4 μmol NH_4^+/g/DM, respectively.

Similar results were obtained for free ammonium levels in treatments under silicon action and control and the silicon did not present interference on this parameter. Ammonium can be absorbed directly in substrate and/or produced by the plants through nitrate reduction in shoots or roots (Harrison *et al*., 2000), where the organic

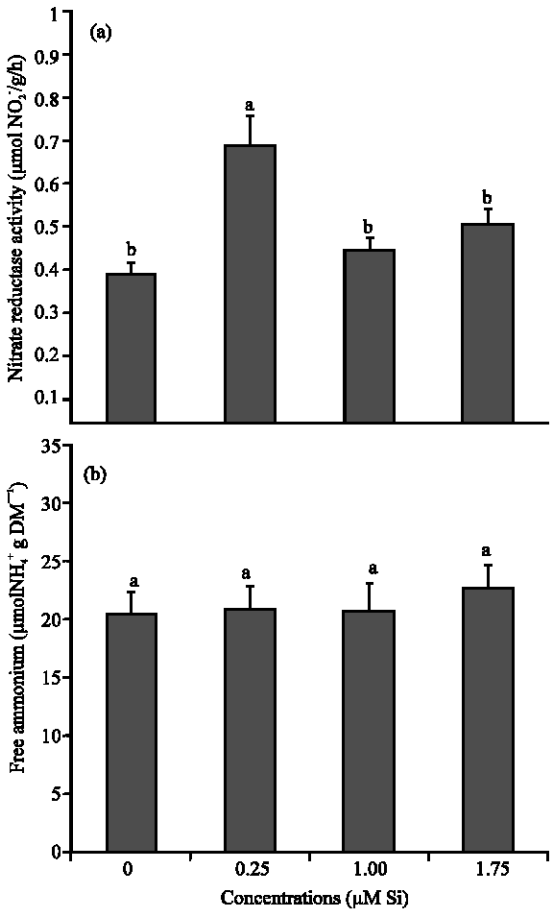

Fig. 2: (a) Nitrate reductase activity and (b) free ammonium in *Capsicum annuum* cv. Vermelho gigante under 4 silicon concentrations (0, 0.25, 1.00 and 1.75 μM Si) and subjected to water deficit. Averages followed by the same letter do not differ among themselves by the Scott-Knott test at 0.05 of probability. The bars represent mean standard errors

ammonium form stimulates the synthesis of essential nitrogen compounds like amino acids and proteins (Wu *et al*., 2007). Similar results on the decrease in free ammonium were reported by Lobato *et al*. (2009b) working with two *Phaseolus vulgaris* L. cultivars submitted to biotic stress.

Si effects on amino acids and proteins: Silicon caused significant changes in total soluble amino acids (Fig. 3a), in which the exogenous application of silicon proportioned a significant maintenance of the amino acids levels in 0.25, 1.00 and 1.75 μM Si treatments, when compared with control treatment.

On total soluble amino acids, we observed that treatments under silicon reduced water deficit effects and

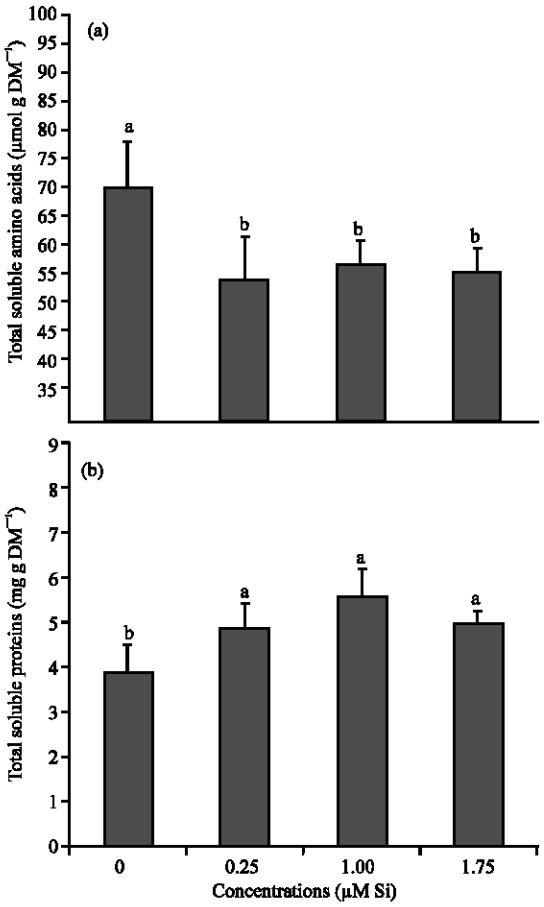

Fig. 3: (a) Total soluble amino acids and (b) total soluble proteins in *Capsicum annuum* cv. Vermelho gigante under 4 silicon concentrations (0, 0.25, 1.00 and 1.75 µM Si) and subjected to water deficit. Averages followed by the same letter do not differ among themselves by the Scott-Knott test at 0.05 of probability. The bars represent mean standard errors

consequently promoted of this nitrogen compound maintenance. Therefore, when vascular plants are frequently exposed to water deficit, they present an increase in the amount of amino acids, due to protein breakdown, aiming at synthesizing specific amino acids to induce osmotic adjustment. Similar results are described by Nath *et al.* (2005) investigating *Capsicum annuum* plants under water restriction, as well as Sankar *et al.* (2007) working with *Abelmoschus esculentus* L. Moench plants.

The total soluble proteins suffered significant changes, according with the statistical test applied. In addition, under concentrations of 0, 0.25, 1.00 and 1.75 µM Si were obtained values of 3.8, 4.8, 5.5 and 4.9 mg/g/DM, respectively. Control plants (0 µM Si) presented lower level, however, silicon under concentrations of 0.25, 1.00 and 1.75 µM Si promoted alleviation of the negative effects in plants induced to water deficiency (Fig. 3b).

For total soluble protein levels the treated plants with silicon were above to control plants, probably due to an increase promoted by silicon on enzymes like superoxide dismutase, peroxidase, catalase and glutathione reductase, which are responsible for avoiding oxidative stress in plants submitted to abiotic stresses (Liang *et al.*, 2003; Gong *et al.*, 2005). Results obtained by Husaini and Abdin (2008) studying wild and transgenic *Fragaria* x *ananassa* Duchesne plants corroborate with the reduction observed in proteins after simulated stress.

Si responses on proline and glycinebetaine: Silicon action promoted improved in proline and the control treatment presented 14.5 µmol/g/DM was significant equal to the treatment under 0.25 µM Si (Fig. 4a). However, the treatments 1.00 and 1.75 µM Si were statistically equal and presented 16.4 and 16.9 µmol g DM^{-1}, respectively.

The increase in proline levels of plants under influences of silicon proved that this element maximizes proline production and other experiments need be performed to explain this fact. Proline is an important amino acid that is over-expressed in tolerant plants to environments under abiotic stress (Turan *et al.*, 2007). These results on proline accumulation in plants exposed to silicon agrees with the study carried out by Gunes *et al.* (2007b) working with *Hordeum vulgare* L. under boron toxicity, as well as the increase in this compound obtained by Lobato *et al.* (2008d), while investigating *Glycine max* L. Merr. plants submitted to water deficiency.

Glycinebetaine levels in pepper plants presented decreases at 14.5, 18.8 and 27.1% in 0.25, 1.00 µM and 1.75 µM Si, respectively, when compared to control (0 µM Si) plants (Fig. 4b). In addition, the concentration under 1.75 µM Si presented better result.

Plants treated with silicon presented lower glycinebetaine rates, when compared to control plants and parameter maintenance is related to applied stress reduction.

Therefore, these results suggest that silicon does not maximize glycinebetaine production, as previously was reported in this on proline results. Ramos *et al.* (2005) reported glycinebetaine accumulation in *Glycine max*

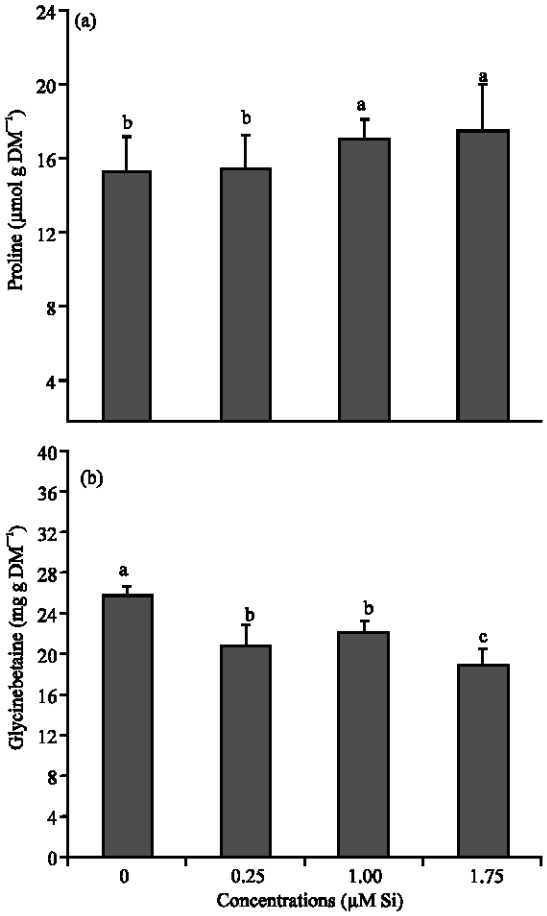

Fig. 4: (a) Proline and (b) glycinebetaine in *Capsicum annuum* cv. Vermelho gigante under 4 silicon concentrations (0, 0.25, 1.00 and 1.75 µM Si) and subjected to water deficit. Averages followed by the same letter do not differ among themselves by the Scott-Knott test at 0.05 of probability. The bars represent mean standard errors

plants under water deficiency, in addition similar results were found by Cha-um *et al.* (2007) working with sensitive and tolerant cultivars of *Oryza sativa* L.

CONCLUSION

This study revealed that silicon attenuated the negative effects promoted by water deficiency on leaf relative water content, stomatal conductance, total soluble proteins, total soluble amino acids and glycinebetaine. Furthermore, the nitrate reductase activity were observed significant benefits only at the concentration of 0.25 µM Si+, however in free ammonium was no obtained improvement after silicon application. Proline level was maximized at concentrations of 1.00 and 1.75 µM Si.

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