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ЕФЕКТ НА СИЛИЦИЯ ВЪРХУ ЕНЗИМНАТА И НЕЕНЗИМНАТА АНТИОКСИДАНТНА ЗАЩИТНА СИСТЕМА ПРИ ХИДРОПОННО ОТГЛЕЖДАНИ КРАСТАВИЦИ (*CUCUMIS SATIVUS* L.) В УСЛОВИЯ НА СОЛЕВИ СТРЕС

EFFECT OF SILICON ON ENZYMATIC AND NON-ENZYMATIC ANTI-OXIDANT DEFENSE SYSTEMS IN HYDROPONICALLY GROWN CUCUMBER PLANTS (CUCUMIS SATIVUS L.) UNDER SALT STRESS

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Abstract

The aim of the study was to investigate the ability of young hydroponically grown cucumber plants (*Cucumis sativus* L.*)* to recover from salt stress through Si-treatment. It was established that exogenous Si enhances the antioxidant capacity of plants, changes the activity of the antioxidant enzymes guaiacol peroxidase (GPX), syringaldazine peroxidase (SPX), the polyphenol content, antiradical activity (DPPH) and lipid peroxidation (LPO).

Key words: antioxidant enzymes, cucumber, lipid peroxidation, salt stress, Si.

INTRODUCTION

Silicon (Si) is the second most abundant element both on the surface of the Earth’s crust and in the soils. Silicon is present as silicic acid $Si(OH)_4$ in the soil solution at concentrations normally ranging from 0,1 to 0,6 mM, roughly two orders of magnitude higher than the concentrations of phosphorus in soil solutions (Epstein, 1999).

The beneficial influence of silicon in decreasing the negative effects of different stress factors like salinity, drought, metal toxicity, fungal infections, pest invasion and many others have been studied. The mitigate effect of Si on salinity has been examined in rice (Yeo et al., 1999), barley (Liang et al., 2003; 2005), cucumber (Zhu et al., 2004), tomato (Al-Aghabary et al., 2005) and other plant species.

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Si has not been considered as an essential element for higher plants, but it has been proved to be beneficial for the healthy growth and development of many plant species (Epstein, 1999; Ma et al., 2001). The beneficial effects of Si are particularly distinct in plants exposed to abiotic and biotic stresses (Ma, 2004). Over last two decades, more extensive and intensive studies have been performed aiming at better understanding of the possible mechanism(s) for Si-enhanced resistance and/or tolerance of higher plants to both abiotic and biotic stresses (Liang et al., 2003).

The addition of Si significantly increases superoxide dismutase (SOD), guaiacol peroxidase (GPX), ascorbate peroxidase (APX), dehydroascorbate reductase (DHAR) and glutathione reductase (GR) activity and decreased electrolyte leakage percentage (ELP), lipid peroxidation (LPO) level and H_2O_2 concentration in salt-stressed cucumber leaves. Si may act to alleviate salt stress in cucumber by decreasing permeability of plasma membranes and membrane lipid peroxidation, and maintaining the membrane integrity and function. A significant increase in antioxidant enzymes of salt-stressed leaves by Si addition suggests that Si may be involved in the metabolic or physiological activity in cucumber exposed to salt stress (Zhu et al., 2004).

The aim of the present study was to investigate the influence of silicon on plant defense system measuring syringaldazine peroxidase (SPX), guaiacol peroxidase (GPX), lipid peroxidation (LPO), polyphenol content and antiradical activity (DPPH).

MATERIALS AND METHODS

Seeds of cucumber, (*Cucumis sativus* L.), cultivar „Gergana" were rinsed thoroughly with distilled water, and germinated on moist filter paper in an incubator at 22-23^{o}C. After 10 days, the seedlings were transferred to plastic containers. Each pot had 4 plants with 5 l of continuously aerated 1/2 Hoagland nutrient solution. Each variant consisted of three replications of 12 plants. Daily photoperiod was 12 h and the temperature was 22-23^{o}C. The pH of the solution was daily adjusted to 6,0-6,5 with 0,01 M KOH and 0,01 M H_2SO_4.

Salinity and silicon treatments were started by adding sodium chloride (NaCl) and potassium silicate (K_2SiO_3) to the nutrient solution immediately after 10-day-old seedlings were transplanted to the nutrient solution. Additional K introduced by K_2SiO_3 was subtracted from KNO_3 and the resultant nitrate loss was supplemented with dilute nitric acid. The initial pH of the nutrient solution after the addition of potassium silicate was adjusted to 6,0 using H_2SO_4 before transplanting.

The experimental design consisted of a control (no added Si and NaCl) and three treatments (50 mM NaCl, 1,5 mM Si, and 1,5 mM Si combination with 50 mM NaCl) were arranged. Analyses of enzyme activity guaiacol peroxidase (GPX), syringaldazine peroxidase (SPX), antiradical activity (DPPH), polyphenol content and lipid peroxidation (LPO) were performed on the 3-th fully expanded leaves after 10 days in hydroponics at the end of experiment.

The total polyphenols were established by the method of Singleton and Rossi (1965) with Folin-Ciocalteu reagent - spectrophotometrically at 765 nm wavelength, and were expressed as galic acid equivalents (mg g^{-1} FW). The DPPH (2,2-diphenil-1-picrylhydrazyl) assay was performed according to Brand-Williams et al., (1995) spectrophotometrically at 515 nm. The reaction mixture was observed against blank, which did not contain extract. Radical scavenging activity was expressed as % inhibition. The activity of syringaldazine peroxidase was determined spectrophotometrically by the method of Imberty (1985). The activity of guaiacol peroxidase was determined spectrophotometrically by the method of Bergmeyer (1974). The lipid peroxidatin (concentration of MDA) was determined according to the method of Heath and Packer (1968).

Statistical analysis of the data obtained was performed using one-way ANOVA (for P<0,05). Based on ANOVA results Tukey's test for the main comparison at 95% confidential level was applied.

RESULTS AND DISCUSSION

Results on fig. 1 present that in leaves of cucumber exposed to salt stress, the activity of enzyme GPX decreased significantly. The addition of Si considerably decreased GPX activity of non-salt stressed leaves by more than 60% compared to the control and increased GPX activity by 10% in variant 50 mM NaCl + Si compared to variant 50 mM NaCl without Si. These results coincided with the results of Zhu et al. (2004). They investigated the antioxidant enzyme activity of cucumber plants and found that the enzyme activity of the Si-treated salt-stressed plants increased significantly at the 10-th day.

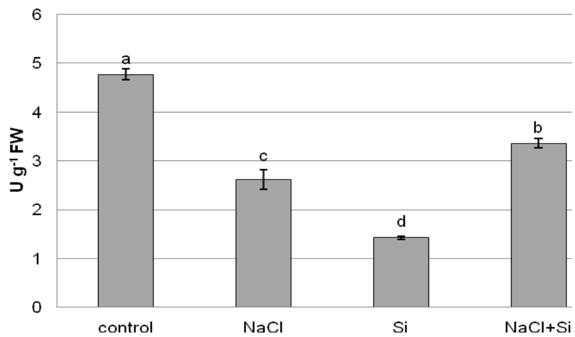

Fig. 1. *Activity of GPX (U g^{-1} FW) in leaves of cucumber plants (Cucumis sativus* L.*): 1 – control; 2 – 50 mM NaCl; 3 – 1,5 mM Si; 4 – 50 mM NaCl + 1,5 mM Si. Data are the mean ± S.D. of three replicates. Different letters in one measure indicate statistically significant differences at P<0.05*

Фиг. 1. *Активност на GPX (U g^{-1} FW) в листа от краставица (Cucumis sativus* L.*): 1 – контрола; 2 – 50 mM NaCl; 3 – 1,5 mM Si; 4 – 50 mM NaCl + 1,5 mM Si. Данните са средни стойности ± S.D. от 3 повторения. Различните букви в едно отчитане показват статистически доказани различия при степен на доказаност P<0.05*

Analysis of the activity of the enzyme syringaldazine peroxidase (SPX) is not widely used to evaluate the effect of silicon on plants under salt stress. The value of the enzyme activity in salt stressed plants decreased by 29% compared to the control. The silicon supply to the salt-stressed plants enhanced the value of syringaldazine peroxidase in the leaves by 40%.

The value of the enzyme is lowest in the leaves of Si-treated non-stressed plants. It is 65 % lower compared to the control (Fig. 2).

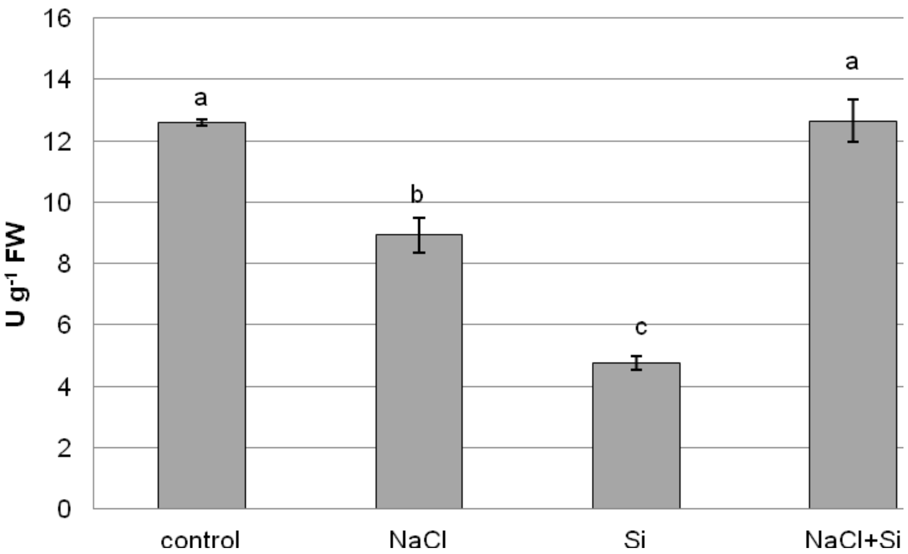

Fig. 2. *Activity of SPX (U g^{-1} FW) in leaves of cucumber plants (Cucumis sativus* L.*): 1 – control; 2 – 50 mM NaCl; 3 – 1,5 mM Si; 4 – 50 mM NaCl + 1,5 mM Si. Data are the mean ± S.D. of three replicates. Different letters in one measure indicate statistically significant differences at P<0.05*

Фиг. 2. *Активност на SPX (U g^{-1} FW) в листа от краставица (Cucumis sativus* L.*): 1 – контрола; 2 – 50 mM NaCl; 3 – 1,5 mM Si; 4 – 50 mM NaCl + 1,5 mM Si. Данните са средни стойности ± S.D. от 3 повторения. Различните букви в едно отчитане показват статистически доказани различия при степен на доказаност P<0.05*

Polyphenols are one of the most important groups of secondary metabolites with anti-oxidative properties. Results in fig. 3 show that the polyphenol content in the leaves of salt-stressed plants increased by 10% in comparison to the control. Si addition decreased significantly the amount of polyphenols in the leaves of salt stressed plants by 16%.

The lowest value of the polyphenol content is present in the leaves of non-stressed cucumber plant supplied with Si. It is 18% lower compared to the control. The tendency is similar in roots (Fig. 3). Hashemi et al. (2010) investigated the effects of silicon nutrition in alleviating salinity stress in hydroponically grown canola (*Brassica napus* L.).

In their research the amount of soluble polyphenols increased significantly in the NaCl treatment in both roots and shoots in canola, which is in line with our results. In their experiment Si nutrition did not have any significant effect on polyphenol content in the roots and shoots.

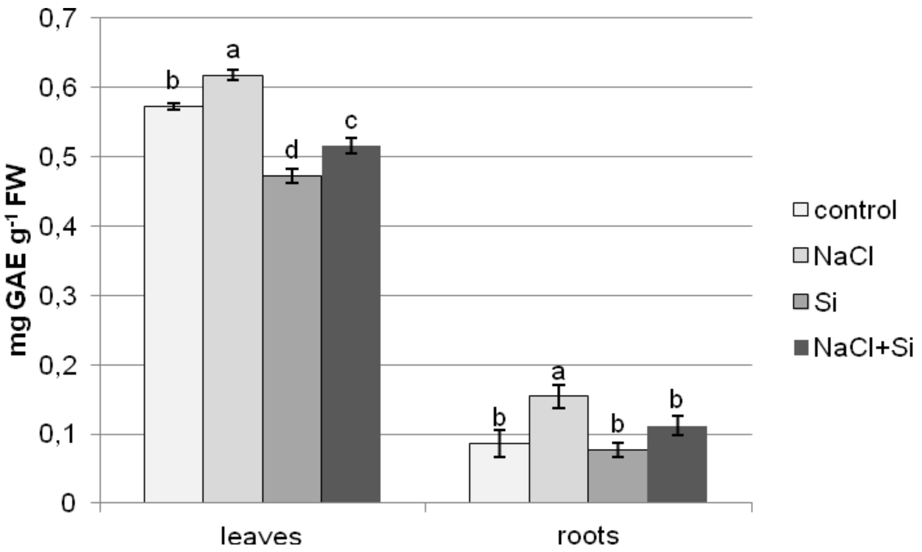

***Fig. 3**. Polyphenol content (mg GAE g^{-1} FW) in leaves and roots of cucumber plants (Cucumis sativus L.): 1 – control; 2 – 50 mM NaCl; 3 – 1,5 mM Si; 4 – 50 mM NaCl + 1,5 mM Si. Data are the mean ± S.D. of three replicates. Different letters in one measure indicate statistically significant differences at P<0.05*

***Фиг. 3.** Съдържание на полифеноли (mg GAE g^{-1} FW) в листа и корени от краставица (Cucumis sativus* L.*): 1 – контрола; 2 – 50 mM NaCl; 3 – 1,5 mM Si; 4 – 50 mM NaCl + 1,5 mM Si. Данните са средни стойности ± S.D. от 3 повторения. Различните букви в едно отчитане показват статистически доказани различия при степен на доказаност P<0.05*

DPPH radical scavenging activity is a measure of non-enzymatic antioxidant activity. Sodium chloride increased the DPPH radical scavenging activity in the leaves of salt-stressed plants compared to the control by 58% and the presence of Si decresed its value in variant NaCl + Si by 38% compared to the salt-stressed plants without Si in nutrient solution (Fig. 4).

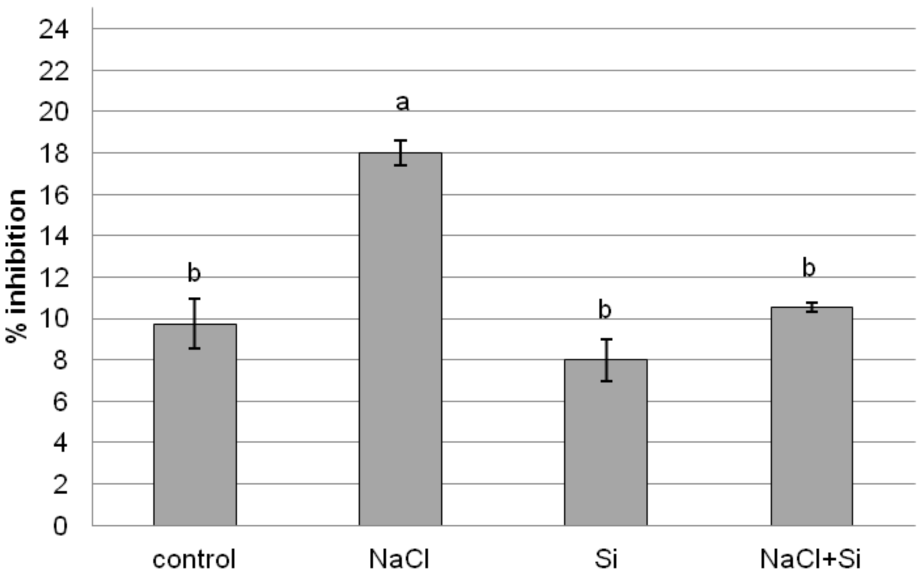

Fig. 4. *DPPH radical scavenging activity (% inhibition) in cucumber plants (Cucumis sativus L.) (leaves): 1 – control; 2 – 50 mM NaCl; 3 – 1,5 mM Si; 4 – 50 mM NaCl + 1,5 mM Si. Data are the mean ± S.D. of three replicates. Different letters in one measure indicate statistically significant differences at P<0.05*

Фиг. 4. *Антирадикалова активност DPPH (% инхибиране) в листа на краставица (Cucumis sativus L.): 1 – контрола; 2 – 50 mM NaCl; 3 – 1,5 mM Si; 4 – 50 mM NaCl + 1,5 mM Si. Данните са средни стойности ± S.D. от 3 повторения. Различните букви в едно отчитане показват статистически доказани различия при степен на доказаност P<0.05*

Malondialdehyde (MDA) is a product of peroxidation of membrane lipids. Under salt stress it can accumulate very fast. That leads to increase in permeability of plasma membrane (Hernandez et al., 1993). In our experiment the content of MDA in the leaves of salt-stressed plants without Si in the solution increased by more than two times in comparison to the non-stressed control plants.

The presence of Si in the nutrient solution of salt-stressed variant decreased the value of MDA by 60%. In variant with Si only the concentration of MDA was 27% lower compared to the control (Fig. 5).

The decrease of lipid peroxidation level of the Si-treated salt-stressed plants coincided with the results of Zhu et al. (2004) in cucumber and Liang et al. (2003) in barley. Silicon addition decreased the permeability of plasma membrane of cells and membrane lipid peroxidation. That improved the ultrastructure of chloroplasts and protected the membrane integrity and functions of salt-stressed cucumber plants (Zhu et al., 2004).

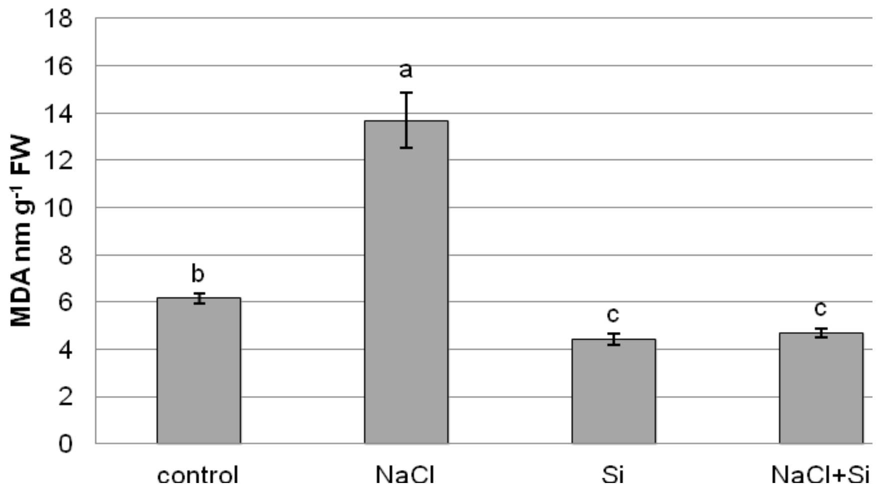

Fig. 5. *Lipid peroxidation (MDA nmol g^{-1} FW) in cucumber plants (Cucumis sativus L.) (leaves): 1 – control; 2 – 50 mM NaCl; 3 – 1,5 mM Si; 4 – 50 mM NaCl + 1,5 mM Si. Data are the mean ± S.D. of three replicates. Different letters in one measure indicate statistically significant differences at P<0.05*

Фиг. 5. *Липидна пероксидация (MDA nmol g^{-1} FW) в листа от краставица (Cucumis sativus* L.*): 1 – контрола; 2 – 50 mM NaCl; 3 – 1,5 mM Si; 4 – 50 mM NaCl + 1,5 mM Si. Данните са средни стойности ± S.D. от 3 повторения. Различните букви в едно отчитане показват статистически доказани различия при степен на доказаност P<0.05*

CONCLUSIONS

1. Silicon supply to the nutrient solution may considerably alleviate the negative effects of salinity: the activity of enzymes GPX and SPX and the polyphenol content increase, while the DPPH radical scavenging activity and the concentration of MDA in leaves decrease.

2. Silicon is probably involved in the metabolism of young cucumber plants exposed to saline stress, enhancing the antioxidant enzyme capacity and protecting the cell membranes from oxidative damage. Silicon improves the rigidity of the cell walls, acts as a mechanical barrier and alleviates the influence of biotic and abiotic stress factors.

REFERENCES

Al-Aghabary, K., Zhu, Z., Shi, Q., 2005. Influence of silicon supply on chlorophyll content, chlorophyll fluorescence and antioxidative enzyme activities in tomato plants under salt stress. J Plant Nutr. 27(12): 2101–2115.

Brand-Williams, W., Cuvelier, M., Berset, C., 1995. Use of a free radical method to evaluate antioxidant activity. Food Science and Technology, 28: 25–30.

Bergmeyer, H., Bernt, E., 1974. MDH-Bestimmung der Aktivitat. In: Methoden der enzymatischen Analyse, Band 1. Weinheim/Bergstrasse: Verlag Chemie GmbH: 649–653.

Epstein, E., 1999. Silicon. Annu. Rev. Plant Physiol. Plant Mol. Biol. 50: 641–664.

Hashemi, A., Abdolzadeh, A., Sadeghipour H., 2010. Beneficial effects of silicon nutrition in alleviating salinity stress in hydroponically grown canola, *Brassica napus* L., plants. Soil Sci Plant Nutr. 56: 244–253.

Heath., R., Packer, L., 1968. Photoperoxidation in isolated chloroplasts. I. Kinetics and stoichiometry of fatty acid peroxidation. Arch Biochem Biophys 12: 189–198.

Hernandez, J., Corpass, F., Gomez, M., del Rio, L., Sevilla, F., 1993. Salt-induced oxidative stress mediated by active oxygen species in pen leaf mitochondria. Physiologia Plantarum 89: 103–110.

Imberty, A., Goldberg, R., Catesson, A., 1985. Isolation and characterization of Populus isoperoxidases involved in the last step of lignin formation. Planta, 164: 221–226.

Liang, Y.C., Chen, Q., Liu, Q., Zhang, W.H., Ding, R.X., 2003. Exogenous silicon (Si) increases antioxidant enzyme activity and reduces lipid peroxidation in roots of salt-stressed barley (*Hordeum vulgare* L.). J. Plant Physiol. 160: 1157–1164.

Liang, Y.C., Zhang, W.H., Chen, Q., Ding, R.X., 2005. Effects of silicon on tonoplast H+-ATPase and H+-PPase activity, fatty acid composition and fluidity in roots of salt-stressed barley (*Hordeum vulgare* L.). Environ. Exp. Bot. 53: 29–37.

Ma, J.F., Miyake, Y., Takahashi, E., 2001. Silicon as a beneficial element for crop plants. In: Datnoff, L., Snyder, G., Korndorfer, G. (Eds.), Silicon in Agriculture. Elsevier Science, New York: 17–39.

Ma, J.F., 2004. Role of silicon in enhancing the resistance of plants to biotic and abiotic stresses. Soil Sci. Plant Nutr. 50: 11–18.

Singleton, V., Rossi J., 1965. Colorimetry of total phenolics with phosphomolybdic phosphotungstic acid reagents. American Journal of Enology and Viticulture, 16: 144–153.

Yeo, A.R., Flowers, S., Rao, G., Welfare, K., Senanayake, N., Flowers, T., 1999. Silicon reduces sodium uptake in rice (*Oryza sativa* L.) in saline conditions and this is accounted for by a reduction in the transpirational bypass flow. Plant Cell Environ. 22: 559–565.

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