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Effect of Silicon on Activity of Antioxidant Enzymes and Photosynthesis in Leaves of Cucumber Plants (*Cucumis sativus* L.)

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Abstract

The effects of exogenous silicon (Si) on changes of photosynthesis and the activities of major antioxidant enzymes such as guaiacol peroxidase (GPOD), siringaldasine peroxidase (SPOD) and antiradical activity (DPPH) as well as the content of polyphenols and photosynthetic pigments were investigated in leaves of young cucumber plants (*Cucumis sativus* L.), cv. Gergana. Plants were grown as a water culture in climatic boxes, under a PPFD of 350 μmol m^{-2} s^{-1}. Five treatments consisting of a control (basic Hoagland nutrient solution without Si) and basic nutrient solution with 0.5 mM Si, 1.0 mM Si, 1.5 mM Si and 2.0 mM Si, were investigated. Plants were grown 15 days and analyses were performed at the end of experiment on the third leaf, which was fully developed. It was established that Si treatment increased photosynthetic activity of leaves. The variant with 1.5 mM Si has shown the highest photosynthetic rate. Activity of main antioxidant enzymes decreased in plant leaves and roots. The content of polyphenols was changed insignificantly in roots and in leaves of Si-treated plants. The content of pigments increased and highest values were established in variant with 1.5 mM Si. These results suggested that exogenous Si application in nutrient solution was useful to increase young cucumber antioxidant capacity and photosynthesis.

Key words: cucumber (*Cucumis sativus* L.), silicon, antioxidant enzymes, photosynthesis

Introduction

Silicon (Si) is the second most abundant element both on the surface of the Earth's rind and in the soils after oxygen (Richmond & Sussman, 2003). Silicon is present as silicic acid 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. Although Si has not been considered as an essential element for higher plants, it has been proved to be beneficial for the healthy growth and development of many plant species, particularly graminaceous plants such as rice and sugarcane and some cyperaceous plants. The beneficial effects of Si are particularly distinct in plants exposed to abiotic and biotic stresses (Epstein, 1999; Ma, 2004).

Increased Si supply improves the structural integrity of crops and may also improve plant tolerance to diseases, drought and metal toxicities (Epstein, 1999; Richmond & Sussman, 2003). For example, Si deposition in the cell walls of root endodermal cells may contribute to the maintenance of an effective apoplastic barrier and thereby improve plant resistance to disease and drought stresses (Lux et al., 2002; Hattori et al., 2005), whilst intra- and extracellular deposition of aluminosilicates in roots and shoots is thought to protect some species from potential Al toxicity (Hodson & Sangster, 1999; Britez et al., 2002; Jansen et al., 2003; Wang et al., 2004).

Numerous studies have shown that Si is effective in controlling diseases caused by both fungi and bacteria in different plant species. For example, Si increases rice resistance to leaf and neck blast, sheath blight, brown spot, leaf scald and stem rot (Datnoff et al., 1997).

Two mechanisms for Si-enhanced resistance to diseases have been proposed. One is that Si acts as a physical barrier. Si is deposited beneath the cuticle to form a cuticle–Si double layer (Fauteux et al. 2005). This layer can mechanically impede penetration by fungi and, thereby, disrupt the infection process. Another mechanism proposed recently is that soluble Si acts as a modulator of host resistance to pathogens. Despite the large number of studies, the effect of different silicon concentrations on physiological state of plants under normal growth conditions is less studied.

The aim of this study was to investigate the effects of different silicon concentrations in nutrient solution on photosynthesis and antioxidant activity of cucumber plants grown in controlled conditions.

Materials and Methods

Young cucumber plants (*Cucumis sativus* L.), cv. Gergana were grown as a water culture in climatic boxes, under a PPFD of 350 μmol m^{-2} s^{-1}. Five treatments consisting of a control (basic Hoagland nutrient solution without Si) and basic nutrient solution with 0.5 mM Si, 1.0 mM Si, 1.5 mM Si and 2.0 mM Si, were investigated. Plants were grown 15 days and analyses of enzyme activity (guaiacol peroxidase-GPOD, siringaldasine peroxidase-SPOD), antiradical activity, polyphenol content and photosynthesis were performed at the end of experiment on the third leaf, which was fully developed.

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 radical) assay was performed according to Blois (1958), which measures the H-donor activity of the extract. The reaction mixture was observed against blank, which did not contain extract and absorbance was measured at 517 nm. Radical scavenging activity was expressed as µmol DPPH quenched by g FM. The activity of syringaldazine peroxidase was determined spectrophotometrically by the method of Imberty (1985). The activity of guaiacol peroxidase was determined spectrophoto-metrically by the method of Bergmeyer (1974). Gas exchange measurements were performed with a portable photosynthetic system LCA-4 (Analytical Development Company, Hoddesdon, UK) equipped with a PLCB-4 chamber. PPFD was 750 μmol m^{-2} s^{-1}, provided by a 500 W incandescent lamp equipped with a reflector. Leaf temperature was 27±2 ºC and ambient CO_2 concentration (Ca) was 370 μmol mol^{-1}. Photosynthetic pigments were determined spectrophotometrically in the same leaf samples, and calculated as in Lichtenthaler (1987).

Each variant was analyzed in four replicates. Statistical analysis of the data obtained was performed using one-way ANOVA (for P<0,5). Based on ANOVA results Tukey's test for the main comparison at 95% confidential level was applied.

Results

Exogenous application of Si by nutrient solution on young cucumber plants had obvious effects. The plants were grown strong and healthy with good leaf appearance and vivid look. The content of pigments increased and the highest values of chlorophyll *a,* chlorophyll *b* and also carotenoids were established in variant with 1.5 mM Si (table 1). These results correlated with the observation that Si treatment increased photosynthetic activity of leaves. The variant with 1.5 mM Si have shown the highest photosynthetic rate (table 2).

Table 1. Content of photosynthetic pigments (mg g^{-1} FM) in leaves of cucumber plants (*Cucumis sativus* L.) grown in different Si concentrations. 1 – control; 2 – 0,5 mM Si; 3 – 1,0 mM Si; 4 – 1,5 mM Si; 5 – 2,0 mM Si.*

Variants	Chlorophyll *a*	Chlorophyll *b*	Chl. *a+b*	Carotenoids	Chl.*a*/Chl.*b*	Chl. /Carot.
1	1,28±0,09	0,51±0,03	1,79±0,12	0,51±0,04	2,53±0,14	3,51±0,21
2	1,36±0,08	0,53±0,02	1,89±0,13	0,54±0,03,	2,56±0,13	3,50±0,20
3	1,52±0,11*	0,66±0,03 *	2,18±0,17 *	0,60±0,04 *	2,37±0,15	3,64±0,24
4	1,57±0,10 *	0,67±0,03 *	2,24±0,18 *	0,61±0,05 *	2,36±0,15 *	3,67±0,23 *
5	1,43±0,09	0,67±0,04 *	2,10±0,16 *	0,58±0,03	2,13±0,14 *	3,62±0,27

* P<0.5

Our results confirmed the observations of other authors worked with silicon. In experiments with tomato plants supplied with silicon Khalid et al. (2005) found that silicon partially raising chlorophyll content, and photochemical efficiency of PSII and thus increased the tolerance of the plants.

Table 2. Leaf gas exchange parameters in leaves of cucumber plants (*Cucumis sativus* L.) grown in different Si concentrations. A- net photosynthetic rate (µmol m^{-2} s^{-1}); E- transpiration intensity (mmol m^{-2} s^{-1}); gs – stomatal conductans (mmol m^{-2} s^{-1}) ; A/E – water use efficiency (µmol $mmol^{-1}$)

Variants	A	E	Gs	A/E
1	18.28±1,32	4.51±0,29	0.79±0,05	4,05±0,23
2	19,36±1,41	4,53±0,27	0,89±0,04	4,27±0,24
3	19,56±1,53	4,66±0,32	0,92±0,05 *	4,20±0,25
4	21,56±1,61 *	4,66±0,35	0,92±0,06 *	4,63±0,31 *
5	21,43±1,48 *	4,67±0,31	0,94±0,06 *	4,59±0,33

*P<0.5

The analysis of activity of the enzyme syringaldazine peroxidase showed statistically significant decrease in the leaves of the treated plants compared with the control plants (Fig. 1A). The lowest value was observed in the highest Si concentration variant. The activity of SPOD in the roots did not show significant change (Fig. 1A). Similar results were observed for the activity of enzyme GPOD in plant leaves and roots with the increasing of Si concentration in nutrient solution (Fig. 1B).

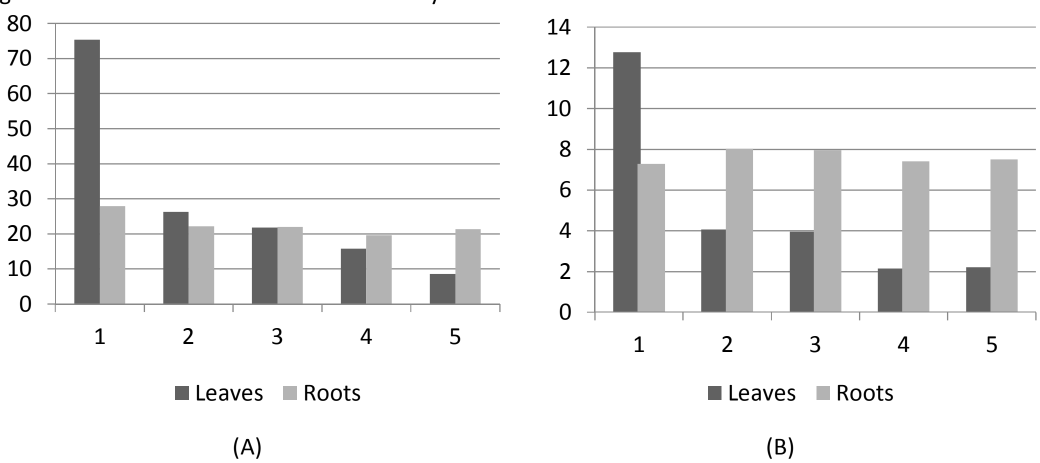

Figure 1. Activity of SPOD (A) and GPOD (B) (U g^{-1} FM) in cucumber plants (*Cucumis sativus* L.) grown in different Si concentrations. 1 – control; 2 – 0,5 mM Si; 3 – 1,0 mM Si; 4 – 1,5 mM Si; 5 – 2,0 mM Si

These results were in accordance with the observations in experiments with tomato plants supplied with silicon (Khalid et al., 2005). In discrepancy of our results Zhujun et al. (2004) reported that silicon supply significantly enhanced the activities of several enzymes such as: SOD, GPOD, APOD, DHAR and GR in salt-stressed leaves of cucumber plants.

The content of total polyphenols was changed significantly in roots but not in leaves of Si-treated plants. The highest values of polyphenols were observed in variants with highest Si concentrations (Table 3). One possible explanation of these results could be that the plants were not stressed.

Table 3. Polyphenol content (mg GAE g^{-1} FM) in cucumber plants (*Cucumis sativus* L.) grown in different Si concentrations. 1 – control; 2 – 0,5 mM Si; 3 – 1,0 mM Si; 4 – 1,5 mM Si; 5 – 2,0 mM Si.

Variants	Leaves	Roots
1	21,36±1,23	14,23±1,08
2	21,28±1,39	14,65±1,11
3	21,76±1,47	14,99±1,21
4	21,38±1,59	15,17±1,35 *
5	21,57±1,62	16,52±1,09 *

* P<0.5

Silicon can prevent abiotic stress in plants. It could be done by polymerized Si which can reinforce the cell walls by physically strengthened the cell wall and inhibiting stressor penetration. The other possibility is that Si may act locally as a signal in triggering natural defence responses in both dicots and monocots, by stimulating the activity of such enzymes as chitinases, peroxidases, polyphenol oxidases, and/or by increasing the production of phenolic compounds, phytoalexins, antimicrobial compounds and systemic stress signals (salicylic acid, jasmonic acid and ethylene) (Pilon-Smits et al., 2009).

The analysis of antiradical activity have shown significant increase in leaves of all treated variants and in roots treated with 1, 1,5 and 2 mM Si (Table 4). These results confirmed the hypothesis that Si-enhanced resistance was due to biochemical modulation of the cell content (Richmond and Sussman, 2003; Ma, 2004).

Table 4. Total antiradical activity (µmol DPPH g-1 FM) in cucumber plants (*Cucumis sativus* L.) grown in different Si concentrations. 1 – control; 2 – 0,5 mM Si; 3 – 1,0 mM Si; 4 – 1,5 mM Si; 5 – 2,0 mM Si.

Variants	Roots	Leaves
1	0,037±0,002	0,126±0,010
2	0,079±0,004 **	0,151±0,012
3	0,087±0,006 **	0,193±0,013 *
4	0,092±0,070**	0,208±0,017 **
5	0,082±0,006 **	0,203±0,01**

P<0.5; ** P<0.1

Several studies in monocots (rice and wheat) and dicots (cucumber) have shown that plants supplied with Si can produce phenolics and phytoalexins in response to fungal infection such as those causing rice blast and powdery mildew (Fawe et al., 1998; Davenport & Tester, 2000; Demidchik, 2002; Belanger et al., 2003; Gad N, 2006; Micó et al., 2008). Silicon is also able to activate some defence mechanisms. For example, in roots of cucumber plants being infected and colonized by Pythium, Si enhanced the activity of chitinases, peroxidases and polyphenoloxydases (Ohnishi, 1990; Cherif et. al.). In rice, differential accumulation of glucanase, peroxidase and PR-1 transcripts were associated with limited colonization by the fungus M. grisea in epidermal cells of a susceptible rice cultivar supplied with Si (Rodrigues et al, 2004; Rodrigues et al. 2005). These biochemical responses are only induced by soluble Si, suggesting that soluble Si might play an active role in enhancing host resistance to diseases by stimulating some mechanisms of the defense reaction (Baker et al., 2000). However, the exact nature of the interaction between the soluble Si and the biochemical pathways of the plant that leads to disease resistance remains unknown, although several possible mechanisms have been proposed (Baker et al., 2000).

According to our results and the information reported from other investigators we could make a speculation that application of Si in nutrient solution to hydroponically grown cucumber plants facilitated their defence systems by increasing of plant non enzyme antioxidant capacity. Besides, Si was capable to increased photosynthetic activity and plant pigment content under non-stressed conditions.

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