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Silicon facilitates manganese phytoextraction by cucumber (*Cucumis sativus* L.)

ABSTRACT

The effect of excess nutrient levels of manganese (Mn, 50 and 100 μM) on the growth inhibition and the appearance of Mn-toxicity symptoms in the leaves was studied in cucumber plants (Cucumis sativus L. cv. Chinese long). Silicon (Si), when supplied as 1.5 mM silicic acid, clearly decreased symptoms of Mn-toxicity despite approximately the same total Mn content in the leaves. In treated plants, Si improves growth and biomass production compared with that of non-Si treated plants. Inert deposition of Si in the leaf cell walls of cucumber (a Si-accumulating species) enhanced cell wall stability. The mechanism of Si protection is proposed to act by Si-induced compartmentation of Mn hence increasing Mn^{2+}-binding sites in the cell wall (e.g. Mn-silicate polymers) finally resulting in decreased toxic free Mn within the plant tissue rather than decrease of Mn uptake. These results suggest that Si nutrition can improve the phytoextraction potential of plants due to enhanced metal tolerance in leaf tissues.

Keywords: *biomass, cell wall, EPR, Mn toxicity, phytoextraction, silicic acid.*

1. INTRODUCTION

Manganese (Mn) is a naturally occurring element that is found in rock, soil, and water. The major pool of manganese in soils originates from crustal sources, with various minor adjuncts including direct atmospheric deposition, wash-off or leaching from plant tissues, and the shedding or excretion of material such as leaves, dead plant, animal material and excrement. The major anthropogenic sources of environmental manganese include municipal wastewater discharges, sewage sludge, mining and mineral processing, emissions from alloy, steel, and iron production, combustion of fossil fuels, and to a much lesser extent, emissions from the combustion of fuel additives. The environmental chemistry of manganese is largely governed by pH and redox conditions. Mn^{2+} dominates at lower pH and redox potential, with an increasing proportion of colloidal manganese oxyhydroxides above pH 5.5. Manganese in soil can migrate as particulate matter to air or water, or soluble manganese compounds can be leached from the soil.

Mn is an essential microelement for plant growth with numerous important physiological functions which specify its nutritional requirements. Different plant species or even varieties within a species have different degrees of tolerance of Mn toxicity. Its concentration around 100 $mg\cdot l^{-1}$ in soil solution leads to phytotoxicity, because Mn ranked as middle-toxic metal [1]. Manganese toxicity is a major factor limiting crop growth causing symptoms of chlorosis, necrotic lesions, and distorted development of the leaves.

Although silicon (Si) is a major constituent of plant tissue, it is not considered to be an essential nutrient for terrestrial plants in general [2]. There are known a numerous beneficial effects of Si in plant resistance against biotic and abiotic stresses [3]. Protective role of Si in conditions of Mn toxicity is confirmed in cucumber [7-10].

The cultivation of plants without soil, in hydroponics - a water solution of nutrients, provides healthier, disease-free plants, with higher yield than grown in soil. Hydroponics provide complete and precise nutritional and water control, ability to manipulate nutrient levels, ability to characterize root growth and morphology, providing a clean root and shoot tissues for chemical, metabolic and molecular analysis. As the most important, hydroponics enables proper conditions to investigate the individual effects of specific elements, because in

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hydroponic systems there are fewer cross-interactions relevant to soil properties [11].

In this work we tested the effect of Si on Mn toxicity in cucumber leaves that leads to decreasing concentration of toxic free Mn^{2+}. Maintaining plant in stabile physiologically state without toxicity symptoms, besides high Mn tissue concentrations, addition of Si significantly improves process of phytoextraction. Therefore, the aim of the presented work was to compare the biomass, visible symptoms and Mn concentration of cucumber plants growing in a nutrient solution with and without Si.

2. MATERIAL AND METHODS

2.1. Plant material and growth conditions

Cucumber (*Cucumis sativus* L. cv. Chinese long) seeds were germinated on filter paper moistened with 2.5 mM $CaSO_4$ and after 5 d the seedlings were transferred to a standard full-strength nutrient solution [9]. After 7-d preculture at optimal Mn concentration (0.5 µM), plants were subjected to different concentrations of Mn (0.5, 50, and 100 µM Mn, respectively) for two weeks. Concomitantly, one half of the plants (+Si) was supplied with 1.5 mM Si as silicic acid prepared by passing Na_2SiO_3 through a column filled with cation-exchange resin (Amberlite IR-120, H^+ form, Fluka). Silicic acid did not change pH of solution after applying. The nutrient solutions were renewed completely every 2 d and continuously aerated. Plants were grown under controlled environmental conditions in a growth chamber with a light/dark regime of 16/8 h, temperature regime of 24/20 ^oC, photon flux density of 300 µmol m^{-2} s^{-1} at plant height, and relative humidity of about 70 %.

2.2. Determination of Mn concentration

After harvest, plants were oven dried at 75 ^oC when dry weight (DW) was measured, ashed at 550 ^oC, and ash was dissolved in 0.5 M HCl for determination of Mn by atomic absorption spectrometry (AAS, Perkin Elmer 403).

2.3. Electron Paramagnetic Resonance (EPR) measurements

Mn^{2+} free and bound form were estimated modifying the method reported earlier [12]. EPR measurements were performed using a Varian E-104A spectrometer (Palo Alto, CA, USA), operating at X-band frequency (9.5 GHz), modulation frequency 100 KHz, modulation amplitude 2 Gauss, scan range 2000 Gauss. EPR conditions were the same in all measurements. The spectra were recorded and analyzed using EW software (Scientific Software International, Inc., Lincolnwood, IL, USA).

2.4 Statistical analysis

Data were subjected to analysis of variance using Statistica 6 (StatSoft, Inc. USA), and means were compared by Mann-Whitney non-parametric test at $p < 0.05$.

3. RESULTS

The development of Mn toxicity symptoms in the form of chlorosis with brown spots and small necrotic regions manifested at higher Mn concentrations is reduced by Si application (Figure 1).

Figure 1 - Effect of root Si supply (1.5 mM) on the development of visible symptoms of Mn toxicity in cucumber leaves

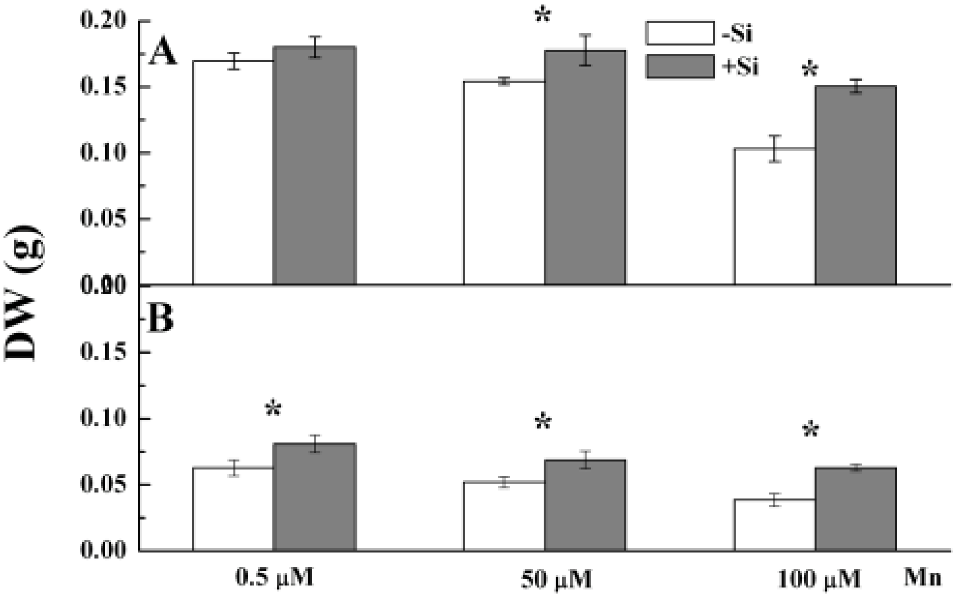

Figure 2 - Effect of root Si supply (1.5 mM) on the biomass of cucumber leaves (A) and roots (B) grown in the nutrient solutions with various Mn concentrations. Data are means of three replications ± SD. Within the same Mn level, bars marked with ***** *indicate a significant difference at $p < 0.05$*

Si stimulates root growth (+Si) at Mn excess. The leaf dry weight of non-Si treated plants (-Si) was slightly lower in control, but Mn excess caused biomass reduction of -Si plants significantly when compare with +Si plants (Figure 2).

Figure 3 shows that -Si roots had significantly higher Mn concentration compared to the +Si roots exceptionally in control. On the contrary, there is no significant differences in leaves Mn concentrations between -Si and +Si plants.

Results of EPR measurements showed that potentially toxic free Mn^{2+} content in cucumber tissue depends on Si supplying in the nutrient solution (Table 1). Applying of Si along with high Mn concentrations in cucumber plants resulted in higher amount of non-toxic wall-bounded Mn^{2+} than those from -Si plants within the same Mn treatment.

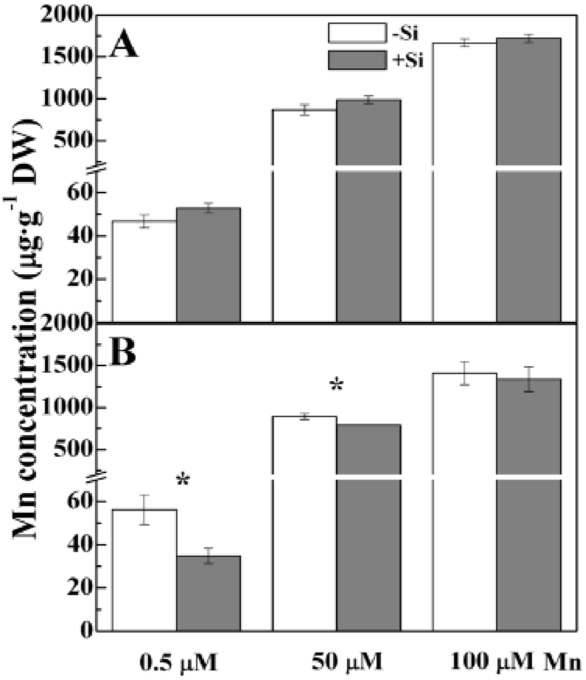

*Figure 3 - Effect of root Si supply (1.5 mM) on the Mn concentrations in cucumber leaves (A) and roots (B) grown in nutrient solutions with various Mn concentrations. Within the same Mn level, bars marked with * indicate a significant difference at $p < 0.05$.*

Table 1 - Effect of root Si supply (1.5 mM) on the ratio of free and wall bound Mn^{2+} concentrations in cucumber roots and leaves grown in nutrient solutions with various Mn concentrations

Mn supply [μM]	Si supply	Root		Leaf	
		Free Mn [%]	Cell wall bound Mn [%]	Free Mn [%]	Cell wall bound Mn [%]
0.5	-Si	31	69	28	72
	+Si	20	80	18	82
50	-Si	11	89	45	55
	+Si	9	91	23	77
100	-Si	17	83	50	50
	+Si	12	88	13	87

4. DISCUSION

There is considerable inter- and intra-specific variation among Mn levels that induce toxicity as well as the symptoms of this toxicity in plant species [13]. The symptoms of manganese toxicity depend on the sensitivity of the plant species, but the cucumber is known as medium-tolerant plants [13]. In the plant tissue, manganese generally tends to accumulate predominantly in the shoot than in the root [14]. The signs of Mn accumulation were observed primarily in the periphery of the oldest leaves which are more sensitive to increased manganese content in them [15]. The appearance of symptoms of toxicity is not directly proportional to the amount of Mn. In the leaves of +Si and –Si plants Mn content is very similar (Figure 3), while the symptoms of toxicity completely absent at +Si plants (Figure 1).

Manganese exists in the environment in two main forms: Mn^{2+} and Mn^{4+}. Transition between these two forms occurs via oxidation and reduction reactions that may be abiotically or microbially mediated [16]. Mn^{2+} form is available for plants and can be readily transported from the soil into the root cells further translocated to the shoots, where it is finally accumulated [17]. A fast Mn transport from roots to shoots [14] was additionally stimulated by Si since the root Mn concentration was lower in +Si compared to –Si plants with simultaneously higher Mn concentration in corresponded leaves (Figure 3).

Biomass comparison showed beneficial effect of Si on growth of cucumber roots in control and under Mn toxicity conditions (Figure 2) as previously reported in other plant species [8, 18. 19]. On the other hand, Si significantly affected leaf biomass production in Mn excess (50 and 100 μM).

In some species (e.g. rice) Si reduces Mn uptake [20], but our results indicated that in cucumber Si supply alleviates Mn toxicity by decreasing the concentration of soluble Mn^{2+} through the enhanced adsorption of Mn to the cell walls (Table 1). Correspondingly, earlier investigations showed that Si redistribute Mn in leaves, foremost by stimulation of Mn binding to the cell wall, thus preventing potentially toxic free Mn^{2+} accumulation in metabolically active leaf parts [10,21].

So far, scientific data revealed that plants uptake and translocate Si in chemical inert form as silicic acid ($Si(OH)_4$) which precipitates in cell wall forming phytoliths [22]. Compartmental analyses of Si-treated cucumber leaves pointed that more than 90% of Si is wall-bounded [23]. Therefore, Si facilitates a stronger cell wall-binding of Mn enhancing Mn detoxification.

The accumulation of Si in the shoots may be related to a number of factors such as transpiration, growth duration, growth rate, but root uptake ability is the most important factor for determining Si accumulation in the shoots. The Si concentration in the xylem sap of cucumber increase fast reaching the maximum after 30 min and remained stable throughout the experiment period [24]. Si pretreatment could have an alleviative effect on Mn toxicity only in the simultaneous presence of high Mn concentrations and their simultaneously availability to the plant. If their availability alternate despite the adopted silicon the symptoms of toxicity will occur [3].

EPR measurements of Mn^{2+} free and cell wall-bound form in snap bean demonstrated that signal intensity of bounded Mn^{2+} is stronger in presence of Si, probably as a result of its complexation to wall compounds [25]. The same pattern was observed in EPR measurements of our cucumber samples, as can be seen in the Table 1. Comparing root and leaf samples far higher concentration of free Mn^{2+} can be observed in leaves, indicating its rapid root to shoot translocation. Hence, our results support the hypothesis of Si protective role in quenching detrimental (inter)actions of free Mn^{2+} within plant tissue.

Accumulation of such high levels of heavy metals is highly toxic and would be lethal for the common nonaccumulator plant. Therefore it is very important to choose the appropriate plant species for the remediation purpose. Most metal hyperaccumulators are slow growing and produce little biomass which severely limits their use for the environment cleanup. Addition of Si could upgrade their biomass production rendering them convenient for phytoextraction.

Blaylock and Huang [26] concluded that the limiting step for metal phytoextraction is the long-distance translocation from roots to shoots. Cucumber exhibits a fast root-to-shoot transport with the significant metal accumulation into the shoot expressing potential capacity for phytoextraction. In this sense, cucumber can be the plant of choice. In cucumber tissue Si enhanced phytoextraction providing Mn-toxicity protection simultaneously enabling biomass production large enough to uptake, translocate and accumulate Mn in leaves for highly effective phytoextraction, while the cell wall-bound Mn fraction could be easily removed by harvesting shoot biomass.

5. CONCLUSION

The Mn concentration of the cucumber tissue correlated positively with the severity of Mn-toxicity symptoms and negatively with the Si supply. This strong correlation is evidence that Si-mediated binding of Mn to the cell wall is the main mechanism of increased Mn tolerance in cucumber. Taking into account this Mn-Si interaction, cucumber, as easily harvestable plant, could have the potential use in the phytoextraction process on sites with light to moderate toxic Mn contamination with the simultaneously Si application.

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IZVOD

SILICIJUMOM OLAKŠANA FITOEKSTRAKCIJA MANGANA KOD KRASTAVCA (*Cucumis sativus* L.)

Efekat visokih koncentracija mangana (Mn, 50 i 100 μM) na inhibiciju rasta i pojavu simptoma toksičnosti mangana u listovima je ispitivan kod krastavca (Cucumis sativus L. sorta Chinese long). Silicijum (Si), kada je dodat hranljivom rastvoru kao 1.5 mM ortosilicijumska kiselina, jasno umanjuje simptome fitotoksičnosti mangana i pored sličnog ukupnog sadržaja Mn u listovima. U tretiranim biljkama, Si poboljšava rast i proizvodnju biomase u poređenju sa netretiranim biljkama. Deponovanje inertnog Si u lignifikovanim ćelijskim zidovima lista krastavaca (koji inače spada u Si-akumulirajuće vrste) povećava stabilnost ćelijskog zida. Predloženi mehanizam zaštite je Si-podstaknuta kompartmentacija Mn povećavanjem mesta akumulacije Mn^{2+} *u ćelijskom zidu (npr. Mn-silikatni polimeri) što konačno rezultira smanjenjem toksičnog slobodnog* Mn^{2+} *u biljnom tkivu. Naši rezultati pokazuju da dodavanje Si u hranljivi rastvor može poboljšati potencijal biljaka za fitoekstrakciju usled povećane tolerancije metala u tkivima listova.*

Ključne reči: *biomasa, ćelijski zid, EPR, toksičnost mangana, fitoekstrakcija, silicijumska kiselina.*

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