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THE RESPONSE OF HYDROPONICALLY GROWN LETTUCE UNDER MN STRESS TO DIFFERENTIATED APPLICATION OF SILICA SOL

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Abstract

A way to alleviate excessive Mn nutrition of plants is through silicon application. The aim of the present study was to examine the effect of different Si concentrations in a nutrient solution and Si plant spraying treatments (in the form of silica sol) on the yielding and nutritional status of hydroponically grown lettuce under Mn-stress. The experiments were conducted under controlled conditions in a phytotron. The influence of the following Si levels in the nutrient solution (5.5 – control; 15.5; 23.25 and 31 mg Si dm⁻³) and foliar sprays (distilled water; Si solution) were investigated. Silicon supplied through fertigation significantly affected the plant’s nutrient status and alleviated the Mn stress, increasing fresh matter production, RWC (Relative Water Content) and the number of leaves per plants, while decreasing the share of dry matter. The Si nutrition did not change the content of Mn in the leaves, but caused a significant increase in N, P, Na, Fe and Si concentrations with a simultaneous decrease of Zn and Cu levels. The content of Ca, Mg and K was relatively stable (except for the treatment with the most intensive Si nutrition). Generally, the concentrations of N, P, K, Ca, Mg, Na and Fe within the tested Si range were higher than in the control, while being lower in the case of Zn and Cu. Overall, the foliar application of Si did not change plant yielding, the number of leaves on plants and most macro- and microelement concentration in leaves, but modified significantly the RWC as well as the Cu and Na content. The dry matter content under Si nutrition was varied. In summary, an effective method to alleviate Mn-stress is to apply silica sol to a nutrient solution used for plant fertigation.

Keywords: silicon, fertigation, foliar spraying, manganese stress, macroelement, microelement, Lactuca sativa L.
Manganese is an essential metallic microelement in plants, but excessive Mn nutrition, apart from causing biochemical disorders, may significantly reduce the biomass production and photosynthesis (Millaleo et al. 2010). In lettuce grown under hydroponic conditions, this effect was found when the concentration of Mn in a nutrient solution increased from 0.5 mg dm$^{-3}$ to 19.2 mg dm$^{-3}$ (Kleiber 2014a). The significantly highest reduction of plant yielding and disorders in nutrient leaf concentrations were determined in combinations where the applied nutrient solution contained 19.2 mg Mn dm$^{-3}$. The Mn stress in plants may be alleviated through silicon (Si) nutrition. Silicon is classified as an essential or beneficial element for some plants, as it may positively influence plant growth/yielding (Ma, Yamaji 2006, Jarosz 2013), especially under stress conditions (Liang et al. 2006), e.g. those induced by a heavy metal (Epstein 1999, Iwasaki, Matsumura 1999). The protective effect of Si against metal toxicity is attributed to the deposition of Si in plant cell walls, where the metal provides binding sites for other metals, thus reducing their apoplastic bypass flow (Ma, Yamaji 2006). Silicon nutrition may also significantly change the nutrient uptake by plants (Epstein 1999, Aziz et al. 2002, Jarosz 2013, Kleiber 2014b). Previous studies focused on the use of different forms of Si as an Mn-stress mitigating element tested on beans (Horst, Marschner 1978), cucumber (Rogalla, Romheld 2002, Maksimović et al. 2007), rice (Horiguchi 1988, Zanão Júnior et al. 2010) or lettuce (Kleiber 2014b).

The aim of the present study was to determine the effect of various silicon concentrations in a nutrient solution and in foliar sprays (in the chemical form of silica sol) on: (i) the content of macro- and microelements (including Mn, and Si) in leaves and (ii) lettuce yields harvested from plants grown under strong Mn-stress (19.2 mg dm$^{-3}$ of nutrient solution).

MATERIAL AND METHODS

Plant-growing experiments (2 independent cycles in 2014) were conducted in a controlled environment growth chamber at the Experimental Station of the Departments of the Faculty of Horticulture and Landscape Architecture, the Poznań University of Life Sciences (Poland). The aim of the study was to assess the effect of varied Si applications on plant response manifested in the nutrient status and yielding of hydroponically grown lettuce (*Lactuca sativa* L. cv. Sunny) under Mn-stress (19.2 mg dm$^{-3}$ of the nutrient solution). The experiments were conducted in a two-factorial design in 8 replications, including the following factors: A – a silicon level in the nutrient solution, B – foliar spraying (B1 – distilled water; B2 – Si solution). During the experiments, the following conditions were maintained: photoperiod,
16 h; temperature, 18°C ± 0.5°C in light and 16°C ± 0.5°C in the dark; RH 70% to 80%; quantum flux density 195 to 205 μmol m⁻² s⁻¹. The light source consisted of high pressure sodium lamps (HPS). The quantum flux density was measured with the SunScan Canopy Analysis System (SS1, Delta-T Devices Ltd., Cambridge, England).

Plants were fertigated using a nutrient solution of the following chemical composition (mg dm⁻³): N-NH₄<10, N-NO₃ 150, P-PO₄ 50, K 150, Ca 150, Mg 50, Fe 3.00, Mn 19.2, Zn 0.44, Cu 0.03, B 0.01, Si 5.5, pH 5.50, EC 1.8 mS cm⁻¹. The following silicon concentrations in the nutrient solution (in mg dm⁻³) were applied: 5.5 (control); 15.5; 23.25 and 31 (factor A). Factor B was foliar spraying (B1 – distilled water; B2 – Si solution: 10 cm³ of 0.1% solution per 1 plant). The source of silicon was silica sol (200 g SiO₂ dm⁻³, Optysil, Intermag Olkus). Plants were sprayed once per cycle, a week after the onset of the experiment. The control treatment consisted in cultivation without additional Si nutrition and with distilled water spraying. During the growth, the following biometrical parameters of plants were measured: the number of leaves, yielding expressed as the mean weight of a lettuce head (g), dry matter (%) and the Relative Water Content (%) – RWC; (Gonzales, Gonzales-Vilar 2001).

On the last day of every cycle, the lettuce heads were collected to be dried and then comminuted. The plant material was mineralized in concentrated sulfuric acid for determinations of the total content of N, P, K, Ca, Mg and Na, in a mixture of nitric and perchloric acids (3:1, v/v) for analyses of total Fe, Mn, Zn and Cu, and in hydrofluoric acid for Si analyses (acc. to Lim, Jackson 1982). After mineralization, the following determinations were performed: N-total using the distillation method according to Kjeldahl in a Parnas Wagner apparatus; P – colorimetrically with ammonia molybdate; K, Ca, Mg, Na, Fe, Mn, Zn, Cu using flame atomic absorption spectroscopy (FAAS, on a Carl Zeiss Jena apparatus), and Si – using flame atomic absorption spectroscopy in acetylene flame with nitrous oxide (on a Varian Spectra 220 FS apparatus). The results were submitted to Anova analysis and the Duncan test (p = 0.05).

RESULTS AND DISCUSSION

The increasing silicon concentrations in the nutrient solution statistically differentiated (p = 0.05) yielding expressed as the mean weight of a lettuce head and as the number of leaves per plant (Table 1). There were no differences in plant performance between Si spraying and H₂O spraying within each treatment. Both an increased dose of Si in the nutrient solution and Si spraying caused a significant increase of the RWC, with the highest mean determined for 31 mg Si dm⁻³, while the lowest for the control (with distilled water sprays). Generally, the Si nutrition reduced the dry matter content in
comparison to the control. Despite the higher biomass production under Si nutrition, symptoms of Mn toxicity on leaves were observed in all the combinations. Overall, the increased Si nutrition significantly influenced the chemical composition of leaves (Tables 2, 3) except for the Mn content. Silicon in the nutrient solution had a significant effect on the content of that element in leaves (Table 3).

Different forms of silicon may be used in plant nutrition, e.g. slow-release Ca- and NH₄-silicates (Górecki, Danielski-Busch 2009), potassium silicate (Lee et al. 2000), choline-stabilized orthosilicic acid (ch-OSA) (Kleiber 2014b) or silica sol (Jarosz 2013). In the present study, two silica sol application methods were tested: in fertigation and in foliar spraying. Si in the form of choline-stabilized orthosilicic acid applied in fertigation had a significant effect on the N status of lettuce, with a simultaneous increase in the P content (Kleiber 2014b). In these experiments, higher P concentrations were recorded in the treatments with increasing doses of Si in the nutrient solution and also in the control with Si spraying. Lee et al. (2000) stated that Si application (up to 3.4 mM Si of a nutrient solution) had a positive effect on

<table>
<thead>
<tr>
<th>Foliar spraying</th>
<th>Si level in nutrient solution (mg dm⁻³)</th>
<th>Plant yield (g head⁻¹)</th>
<th>Number of leaves per plant</th>
<th>RWC</th>
<th>DM (%)</th>
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Key for Tables 1-3: within rows and columns (separately), means marked with different capital letters differ significantly at \( p = 0.05 \); within rows and columns, means marked with different small letters differ significantly at \( p = 0.05 \).
the P uptake, with no such effect occurring in the case of foliar Si spraying. Generally, the content of P in lettuce leaves achieved in our experiment was higher than reported by KariMaei et al. (2004) and Gül et al. (2008), while resembling the results reported by Matraszek et al. (2002). Phosphorus is an essential component of high energy compounds (e.g. ATP), so its growing content in leaves may have been one of the reasons for an improved plant performance (Table 1).

In this study, an upward trend was observed for the leaf K content, which peaked in plants with the most intensive silicon nutrition (Table 2). A similar phenomenon was described by Kleiber (2014b) when doses of

<table>
<thead>
<tr>
<th>Foliar spraying</th>
<th>Si level in nutrient solution (mg dm⁻³)</th>
<th>control</th>
<th>15.5</th>
<th>23.25</th>
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</table>

Table 2

The effect of silicon nutrition on concentrations of macroelements and sodium in lettuce leaves (g kg⁻¹ DM)
0.21-0.42 mg dm\(^{-3}\) Si were applied. Liang et al. (2006) stated that salt-stressed plants were characterized by an increasing K content and reduced osmotic stress. The recorded Ca concentrations were similar to those reported by Kleiber (2014\(b\)), whereas an opposite trend for calcium concentrations was found by Chen et al. (2011) and Jarosz (2013). Kleiber (2014\(b\)) found similar amounts of Na in lettuce leaves, and claimed that Si did not influence that element. In the present study, a marked albeit statistically non-significant upward trend was recorded for the Mg content (Table 2), which could improve the process of photosynthesis. Similarly, Maksimović et al. (2007) stated that silicon may positively influence the content of chlorophyll in plants.

In the current experiments, there was no significant effect of Si on the Mn status of plants (Table 3). A similar result was shown by Kleiber (2014\(b\)) for lettuce’s response depending on Si application. In Horiguchi’s opinion (1988), Si application enhances the internal tolerance to an excessive Mn

<table>
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<th>Si level in nutrient solution (mg dm(^{-3}))</th>
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content in the tissues with a simultaneous decreasing content of that micro-
element in plant. Si-treated plants contain less Mn located in the symplasts
(<10%) and more Mn bounded to the cell walls (>90%) when compared with
non-Si-treated plants (about 50% in each compartment); as a result, Mn is
less available and therefore less toxic (Rogalla, Römheld 2002). Silicon allevi-
ates Mn toxicity in rice not only by reducing Mn uptake by plants but also
by increasing the internal tolerance to an excessive amount of Mn in the
tissues (Horiguchi 1988). Zanão Júnior et al. (2010) showed that with Si added
to a nutrient solution, the concentration of Mn in leaves decreased, which
was paralleled by an increase of its content in roots, hence the toxic effect of
Mn on plants was limited. A similar experiment to ours was conducted by
Maksimović et al. (2007), who studied the effect of Si supplied to hydroponi-
cally grown cucumber with an increasing Mn level. High Mn nutrition in-
duced both growth inhibition of the whole plant and the appearance of
Mn-toxicity symptoms in leaves. The application of Si alleviated Mn toxicity
by increasing the biomass production. Although the total Mn concentration
in the leaves did not differ significantly between +Si and −Si plants, symp-
toms of Mn toxicity were not observed in Si-treated plants. Maksimović et al.
(2012) studied the response of cucumber grown in nutrient solutions with
adequate (0.5 mM) or excessive (100 mM) Mn concentrations and with or
without Si supplied, concluding that the apoplastic concentration of free Mn^2+
and \( \text{H}_2\text{O}_2 \) in high Mn-treated plants was significantly reduced by the Si
treatment. Similarly to our study, Iwasaki et al. (2002) found no significant
differences in the Mn content of plants under Si nutrition.

A positive effect of Si on the Fe content was observed (Table 3). In a pre-
vvious study, Kleiber (2014b) showed an opposite trend for that micronutrient
in leaves under increasing doses of ch-OSA. Silicon levels did not have a
significant effect on the content of Fe in cucumber leaves (Jarosz 2013). In
contrast, silicon significantly affected Zn in lettuce. A similar trend was
found in the case of choline-stabilized orthosilicic acid application (Kleiber
2014b). Zn levels in plants under Si nutrition reported by Jarosz (2013) re-
semble our findings, whereas Lee et al. (2000) found no effect of Si on the Zn
uptake. Unlike the results of Kleiber (2014b) and Jarosz (2013), the current
experiment showed that both root and foliar Si application significantly re-
duced the Cu content in leaves.

The highest Si uptake appeared in the treatment with the most inten-
sive Si nutrition (Table 3). Silicon uptake depends on the concentration of
that element in the root zone (Lee et al. 2000). Fruits of cucumber fed with
Si contained higher Si concentrations than the control in a study by Jarosz
(2013). Also, slow-release silicates contributed to an increased Si content in
cucumber leaves and fruits (Görecki, Danielski-Busch 2009). A higher con-
tent of Si in sunflower leaves in response to Si nutrition was found by
Kamenidou, Cavins (2008), while Romero-Aranda et al. (2006) reported an
increase in the Si concentration in shoots of tomato in the presence of
2.5 mM Si.
Excessive Mn nutrition significantly reduces lettuce yielding (Kleiber 2014a). Maksi\v{m}ovi\v{c} et al. (2007, 2012) reported that positive symptoms of Si application under Mn-stress are manifested by higher biomass production. In this study, that silica sol as a source of available Si was found to be able to alleviate Mn stress; however, a statistically significant positive effect was observed only in the case of Si application to the nutrient solution. A positive effect of Si on lettuce yielding was also found by Re\v{s}ende et al. (2007) and Kleiber (2014b). Similar results have also been reported for other species (Rogalla, Romheld 2002, Zhu et al. 2004, G\'orecki, Danielski-Busch 2009, Zan\'ao J\'unior et al. 2010).

The key mechanisms of Si-mediated alleviation of abiotic stresses in plants are very complicated (Liang et al. 2006) and include the stimulation of antioxidant systems in plants, or complexation or co-precipitation of toxic metal ions with Si. Silicon has a positive effect on the content of chlorophyll (Maksi\v{m}ovi\v{c} et al. 2007) and reduced transpiration rates when compared with untreated plants (Lu, Cao 2001). Moreover, an enhanced plant growth owing to the improved leaf photosynthesis and root activity has been reported, along with the alleviation of osmotic stress by reducing transpiration and/or improving water retention (Liang 2008). Silicon stimulates the plant's antioxidant defence (Neumann, zur Nieden 2001, Shi et al. 2005), reduces lipid peroxidation and improves plasma membrane and tonoplast structure, integrity as well as vital functions (Liang 2008). Two factors are involved in the mechanism of the Si-enhanced Mn tolerance in cowpea: (1) decrease in the concentration of soluble apoplastic Mn through the enhanced adsorption of Mn on the cell walls, and (2) possible detoxification of apoplastic Mn through increased concentrations of the soluble Si in the apoplast (Iwasaki et al. 2002). Agarie et al. (1998) found that the application of Si in a nutrient solution reduced the rate of transpiration through stomatal pores of rice leaves, while Schmidt et al. (1999) stated that Si spraying stimulated antioxidant superoxide dismutase activity and increased photosynthetic capacity and the chlorophyll content in plants, especially under a high fertilizer regime. Silicon assists the incorporation of inorganic phosphate into ATP, ADP and sugar phosphates in sugarcane (Marschner 2002), which could explain the increased phosphorus content in lettuce associated with increasing Si nutrition (Kleiber 2014b). Silicon may also modulate the metabolism and utilization of phenolic compounds, mainly at the leaf level, which is most probably the consequence of the formation of Si-polyphenol complexes (Maksi\v{m}ovi\v{c} et al. 2007). Horiguchi (1988), when studying the effects of Si (50 ppm SiO$_2$) on the alleviation of Mn toxicity (0.32-100 ppm Mn) in rice, found that Si content in the top parts of +Si plants was higher than in Si-untreated plants for all the levels of Mn tested in the solution. Also transpiration rates of the Si untreated plants were higher for all the Mn levels in the solutions. In the case of higher Mn concentrations, the increased Mn concentration in the roots induced by the Si treatment was less pronounced than the decrease in Mn amounts in the plant tops. Peroxidase activities in both the tops and roots incre-
ased with an increase in Mn nutrition; on the other hand, the peroxidase activity of the +Si plants was slightly lower than that of the -Si plants.

CONCLUSIONS

The experiments conducted on lettuce grown under manganese stress showed that silica sol application (as a source of available Si) to a nutrient solution significantly improved the growth and yielding of plants, whereas foliar spraying with the same Si form did not change the plant’s status. The increasing Si nutrition significantly raised the RWC, which paralleled a reduction of DM of leaves, with both parameters affected by the increasing application doses of Si to both the root zone and leaves. Silicon nutrition significantly changed the nutrient status of plants. The alleviation effect of Si was not related with a decrease in the manganese content, but caused a significant increase of the Si content in leaves. Generally, the uptake of N, P, K, Ca, Mg, Na and Fe within the tested Si range was higher than that for Si-0, while being lower for Zn and Cu. Foliar application of Si changed significantly the RWC and Na concentration. The dry matter content was varied in plants given this type of Si nutrition. Thus, an effective method to alleviate Mn-stress seems to be through an application of silica sol to a nutrient solution.

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