Acta Biologica Hungarica 59 (4), pp. 479–487 (2008)
DOI: 10.1556/ABiol.59.2008.4.8

THE EFFECT OF SILICON ON THE SYMPTOMS OF MANGANESE TOXICITY IN MAIZE PLANTS

Zlatimira Stoyanova,[1] Ekaterina Zozikova,[1] Charlotte Poschenrieder,[2] J. Barcelo[2] and Snejana Doncheva[1]*

[1] Department of Mineral Nutrition and Water Relations, Institute of Plant Physiology, Bulgarian Academy of Sciences, Acad. G. Bonchev Str., Bl. 21, 1113 Sofia, Bulgaria
[2] Laboratorio de Fisiologia Vegetal, Facultad de Ciencias, Universidad Autonoma de Barcelona, E-08193 Bellaterra, Spain

(Received: April 24, 2007; accepted: October 4, 2007)

The effect of exogenously applied silicon (Si) on plant growth, lipid peroxidation, total phenolic compounds and non-protein thiols was studied in two maize varieties (*Zea mays* L. vars. *Kneja 605, 434*) differing in sensitivity to excess manganese (Mn). Based on the density of brown spots per leaf area and relative shoot weight (RSW) used to define Mn tolerance var. *Kneja 434* was found to be more Mn-tolerant than *Kneja 605.* The lipid peroxidation level and total phenolic compounds were enhanced with increasing Mn concentration in the nutrient solution. In addition, the Mn-sensitive var. *Kneja 605* with markedly expressed first visible Mn toxicity symptoms had higher levels of total phenolic acids than var. *Kneja 434* thus supporting the hypothesis that a stimulating effect of Mn on phenol content reflected rather a stress response to Mn excess than a tolerance mechanism. In contrast, non-protein SH content increased to a higher extent in the Mn-tolerant var. *Kneja 434.* The increased amount of non-protein SH compounds was accompanied by a much stronger oxidative stress in the Mn-sensitive plants when compared with the Mn-tolerant variety, thus suggesting that non-protein SH compounds may play a role in Mn tolerance in maize. The addition of silicon (Si) reduced the density of brown spots per leaf area as well as lipid peroxidation level and improved plant growth in Mn-treated plants.

Keywords: Manganese toxicity – silicon – phenolic compounds – lipid peroxidation – non-protein thiols

INTRODUCTION

Manganese is a plant micronutrient which depending on its content in the soil, pH redox potential, soil moisture, microbial activity, extreme climatic conditions (water logging, dry, hot conditions) [29, 30] can achieve levels that are toxic for plants [8, 18]. Mn toxicity is one of the most limiting factors for crop production in acid soils [4, 11]. The toxic effects of heavy metals, both essential and non-essential elements,

*Corresponding author; e-mail: doncheva@obzor.bio21.bas.bg

have been linked to the production of free radicals [5, 14]. Oxidation of Mn^{2+} and phenolic compounds in the apoplast by peroxidase to highly reactive Mn^{3+} and phenoxiradicals has been proposed to be a key reaction leading to Mn toxicity. These reactive species are responsible for the formation of brown depositions in cell wall [17, 19]. Plants posses an array of hydrophilic and lipophilic antioxidant such as glutahtione, ascorbic acid, phenolic compounds and the carotenoids. The reduced forms of these compound together with antioxidant enzymes are involved in scavenging reactive oxygen species. For example, in plant systems polyphenolics can act either as antioxidants for detoxification of the active oxygen species caused by heavy metals [14, 27] or they can function as metal chelators [20]. Considerable amounts of non-protein thiols are present in plants that function as antioxidants by keeping other redox-active molecules in a reduced state [12]. However, whether the tolerance to Mn toxicity can be accompanied by increased antioxidant capacity of tolerant genotypes is not clear [13]. In previous experiments [7] we have characterized the level of ascorbate content in leaves accumulating Mn in excess and the effect of Si pretreatment to alleviate the symptoms of toxicity. Our results showed that the tolerant genotype may contain higher ascorbate levels under Mn stress than the sensitive one, which was in accordance with data reported for common bean [13]. However, application of Si increased the tolerance to Mn without increasing the leaf ascorbate redox status.

In the present study, with the aim of obtaining more information about Mn tolerance, we investigated the effect of Mn in the presence or absence of Si on the lipid peroxidation in relation to the content of phenolics and non-protein SH groups of two maize varieties with different sensitivity to Mn.

MATERIALS AND METHODS

Five-day-old maize seedlings vars. *Kneja 605* and *Kneja 434* were grown hydroponically in continuously aerated nutrient solution in a greenhouse under natural light. The basic nutrient solution contained (μM): 200 $CaSO_4 \cdot 2H_2O$, 100 $MgSO_4 \cdot 7H_2O$, 400 KNO_3, 300 NH_4NO_3, 5 $MnSO_4 \cdot H_2O$, 0.38 $ZnSO_4 \cdot 7H_2O$, 0.16 $CuSO_4 \cdot H_2O$, 10 Fe-EDTA, 5 $NaH_2PO_4 \cdot H_2O$, 8 H_3BO_3, 0.06 $(NH_4)Mo_7O_{24} \cdot H_2O$. Half of plants received this nutrient solution (–Si), while the rest was exposed to a solution supplemented with 1 mM Si (+Si). Silicon was supplied at a concentration of 1 mM as a silicic acid got by passing sodium silicate through a H^+ loaded Dowex 50 Wx 8 cation exchange resin [24]. After 72 h in –Si or +Si solutions, the plants were transferred to a new solution with same composition as above without Si but supplemented with 200 or 500 μM Mn as $MnSO_4$. Control plants received the basic nutrient solution containing 5 μM Mn throughout the experiment. The second leaves were harvested for analyses 5 days after treatment. The nutrient solution was changed twice a week.

Growth was determined by the relative root weight (RRW), and relative shoot weight (RSW).

$$\text{RRW or SRW} = \frac{\text{root or shoot dry weight treated}}{\text{control root or shoot weight control}} \times 100$$

For the quantification of the visible Mn toxicity symptoms the area of brown spots was counted in the second leaf and was expressed as percent from the total leaf area.

The concentration of total phenolic compounds in leaves was determined using the Folin–Ciokalteu reagent [31] after alcohol extraction. The absorbance was read at 730 nm. Chlorogenic acid was used as a standard to calculate the concentration of soluble phenolics.

Lipid peroxidation level was estimated using the 2-thiobarbituric acid (TBA) test for determination of MDA (malondialdehyde) content. The specific absorbance of products and non-specific background absorbance was read at 532 and 600 nm. The MDA concentration was calculated using the extinction coefficient 155 $mM^{-1} \cdot cm^{-1}$ [16]. Non-protein SH groups were determined as described by Edreva and Hadjiiska [9] using Ellman's reagent. The level of non-protein SH-groups was calculated using $\varepsilon = 13{,}600\ M^{-1} \cdot cm^{-1}$ for the reaction product.

Data are expressed as means ± SE where $n = 6$. Comparison of means was performed by the Fisher LSD test ($P \leq 0.05$) after performing multifactor ANOVA analysis.

RESULTS AND DISCUSSION

Relative shoot weight (RSW) and relative root weight (RRW) were used to evaluate the response of maize plants to Mn excess. Both RSW and RRW of *Kneja 434* were significantly higher compared with *Kneja 605* both at 200 and 500 μM Mn (Table 1). The RSW of *Kneja 434* exceeded 70% at 200 μM Mn which is the value used to define Mn tolerance in a hydroponics system. Shoot dry weight of the *Kneja 605* was about 60% or below this value, thus indicating that this variety was Mn sensitive [25]. Based on these parameters, *Kneja 434* could be defined as more tolerant than *Kneja 605*. Si supply increased the dry matter of both shoots and roots and enhanced the tolerance of Si/Mn-treated plants compared with Mn-treated plants.

First visible Mn toxicity symptoms in maize plants appear as light-brown spots on old leaves. At all Mn concentrations tested Mn-sensitive *Kneja 605* plants showed a higher density of brown spots per leaf area than Mn-tolerant *Kneja 434* plants (Table 2). Addition of Si prevented the expression of Mn toxicity symptoms in Mn-tolerant plants whereas in Mn-sensitive plants the visual symptoms at 200 μM Mn were significantly reduced at 200 μM Mn.

Evidence has been provided that the Mn toxicity is linked to the formation of phenoxy radicals and highly toxic Mn^{III} [19]. These reactive species might induce further redox reactions of several apoplastic components such as the plasma membrane, e.g. lipid peroxidation [18]. MDA concentration was analyzed as a measure of cellular accumulation of lipid peroxidation products. There were no differences in MDA levels between Mn-tolerant and Mn-sensitive control plants. Treatment with increas-

Table 1
Dry weight of shoots and roots (g plant^{-1}), and relative (%) shoot weight (RSW) and root weight (RRW) of maize plants growing in nutrient solution with different Mn concentrations in the presence or absence of Si

Mn level (μM)	Shoots (g plant^{-1})	Roots (g plant^{-1})	RSW (%)	RRW (%)
Kneja 434 (Mn-tolerant)				
5 (control)	0.264±0.00171^{c}	0.234±0.00516^{c}	100	100
5+Si	0.265±0.00129^{c}	0.247±0.00275^{d}	100	105
200	0.258±0.00126^{ab}	0.220±0.00707^{b}	97.7	94
200+Si	0.259±0.00171^{b}	0.229±0.01036^{c}	98.1	97.8
500	0.256±0.00166^{a}	0.205±0.00206^{a}	96.9	87.6
500+Si	0.260±0.00263^{b}	0.207±0.00141^{a}	98.4	88.5
LSD ($P \leq 0.05$)	0.0025	0.0078		
Kneja 605 (Mn-sensitive)				
5 (control)	0.349±0.00294^{g}	0.193±0.00061^{g}	100	100
5+Si	0.391±0.0034^{h}	0.204±0.00112^{h}	112	105.7
200	0.179±0.00359^{b}	0.120±0.00162^{c}	51	62.2
200+Si	0.223±0.0031^{d}	0.131±0.00261^{d}	63.8	67.9
500	0.155±0.0015^{a}	0.101±0.00036^{a}	44.4	52.3
500+Si	0.184±0.0015^{c}	0.108±0.00021^{b}	52.7	55.9
LSD ($P \leq 0.05$)	0.0038	0.0034		

Different letters indicate significant differences assessed by Fisher LSD test ($P < 0.05$) after performing ANOVA multifactor analysis.

Table 2
Manganese toxicity symptoms (the area of brown spots per leaf area) on the second leaf of maize plants growing in nutrient solution with different Mn concentrations in the presence of Si (+Si) or absence (–Si)

	Manganese concentration (μM)							
	5 (control)		50		200		500	
	–Si	+Si	–Si	+Si	–Si	+Si	–Si	+Si
Kneja 605 (Mn-sensitive)	–	–	+	–	++	+	++++	++
Kneja 434 (Mn-tolerant)	–	–	–	–	+	–	++	+

– none < 5%;
+ insignificant < 10%;
++ strong < 20%;
++++ severe > 30%.

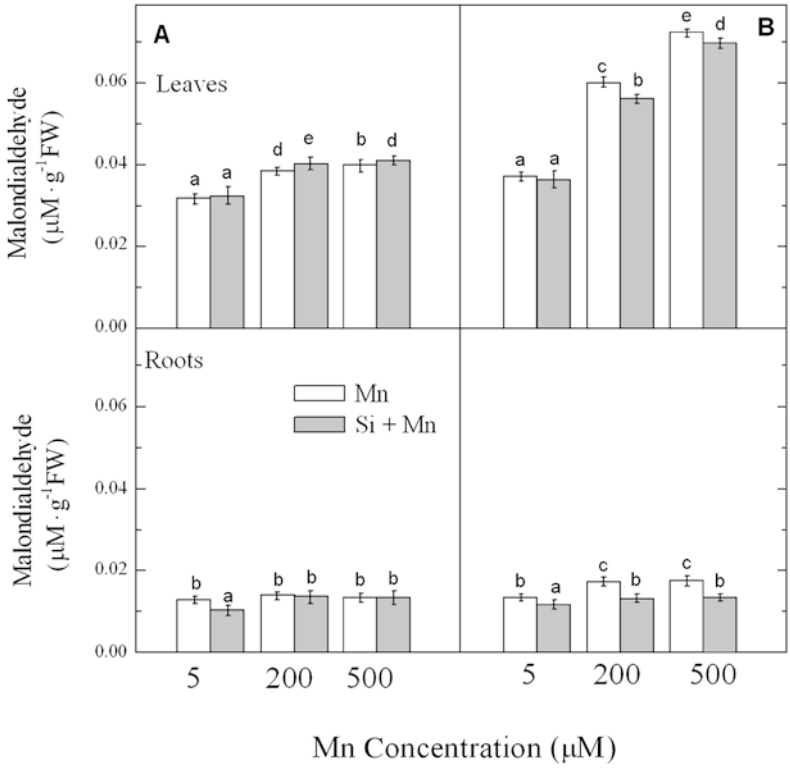

Fig. 1. Effect of increasing Mn concentrations (5 – 500 μM) in the presence or absence of Si on the lipid peroxidation in the leaves and roots of maize plants of vars. *Kneja 434* (A) and *Kneja 605* (B). Values are means ± SE n = 6. Different letters indicate significant differences assessed by Fisher LSD test ($P < 0.05$) after performing ANOVA multifactor analysis

ing concentrations of Mn caused a linear enhancement of MDA in the leaves of Mn-sensitive plants compared with control plants (Fig. 1B). Mn excess affected slightly MDA content in the leaves of Mn-tolerant plants (Fig. 1A) as well as the roots of both varieties (Fig. 1A, B). Peroxidation of cell membranes affects severely its functionality and integrity and can produce irreversible damage to the cell function [15]. However, exogenous Si reduced MDA concentration in Mn-stressed plants indicating that Si can alleviate the oxidative stress caused by Mn toxicity (Fig. 1). On the other hand, it was reported that Si enhanced the stability of lipids in cell membranes, suggesting that Si prevented the structural and functional deterioration of cell membranes [1]. Therefore, Si may play a role in maintaining membrane integrity and stability upon exposure of plants to environmental stress [22]. Plants possess an antioxidant defense system comprised of enzymatic and non-enzymatic components, which normally maintain reactive oxygen species balance within the cell. Phenolic compounds act as non-enzymatic antioxidants in protective mechanisms including both

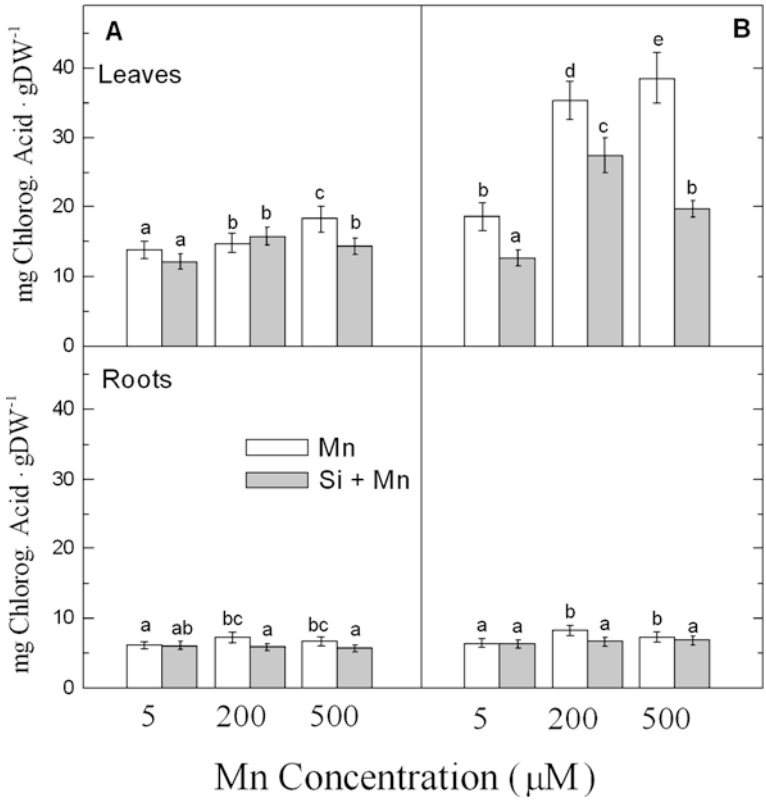

Fig. 2. Effect of increasing Mn concentrations (5 – 500 μM) in the presence or absence of Si on the total phenolic compounds in the leaves and roots of maize plants of vars. *Kneja 434* (A) and *Kneja 605* (B). Values are means ± SE n = 6. Different letters indicate significant differences assessed by Fisher LSD test ($P < 0.05$) after performing ANOVA multifactor analysis

free radical scavenging and chelating and have been shown to be more effective antioxidants *in vitro* than vitamins E and C on a molar basis [26]. In plant cells phenolics can form an antioxidant system equivalent to that of ascorbate [28]. Total soluble phenolics in Mn-sensitive leaves increased with increasing Mn concentrations in the nutrient solution. At 500 μM Mn they reached a value twofold higher compared with the control (Fig. 2B). In the leaves of *Kneja 434* the changes in the concentrations of total phenolics due to Mn treatment were less expressed compared with the Mn-sensitive plants (Fig. 2A).

On the other hand, the control plants of Mn-sensitive *Kneja 605* contained higher concentrations of soluble phenolics in leaf tissues as compared with Mn-tolerant *Kneja 434*. These data suggest that positive correlation between the content of total phenolics and Mn tolerance is not approvable [19]. No changes in phenolics content

were observed in the roots of both tolerant and sensitive plants following Mn treatment. A significant decrease in the level of total phenolics was measured in the leaves of Mn-sensitive plants after Si pretreatment (Fig. 2B). The level of total phenolic compounds in tolerant plants was decreased only at the highest Mn concentration applied (Fig 2A). On the other hand, total phenolics concentration in Mn-stressed roots was not affected by Si treatment. Beckman [2] pointed out that phenolic acids are synthesized by plants in response to physical injury infection or other stresses and they are often stored primarily in the apoplast or in the vacuole, strategically playing either a signalling or direct role in defense reactions. In agreement with the above data we established that the level of total phenolic acids was strongly increased only in maize plants with markedly expressed Mn toxicity symptoms. On the other hand, this result supported the hypothesis that a stimulating effect of Mn on phenol content reflected rather a stress response to Mn excess than a tolerance mechanism [10].

The content of non-protein SH compounds in both tolerant and sensitive plants was significantly enhanced under Mn excess (Fig. 3). It is known that non-protein SH compounds are important factors determining heavy metal tolerance [3]. The pool of non-protein SH compounds include not only glutathione, which is the main non-protein SH (the substrate for phytohelatin biosynthesis and for keeping the ascorbate in reduced form in the ascorbate/glutathione cycle), but also other SH groups [3, 6, 12]. They have been demonstrated that induction of phytochelatins in the presence of heavy metals coincided with a transient decrease in the levels of glutathione [6]. However, Mn-induced phytochelatin synthesis has not been yet observed [26]. In our experiments, the increased amount of non-protein SH compounds due to Mn treat-

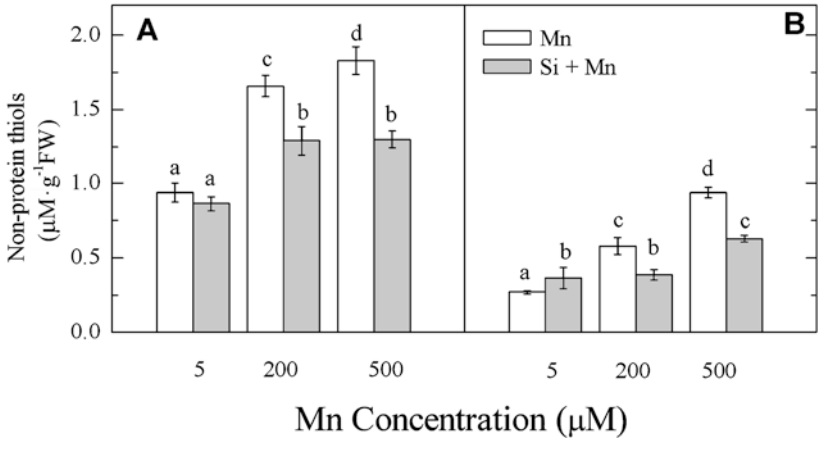

Fig. 3. Effect of increasing Mn concentrations (5, 200 and 500 μM) in the presence or absence of Si on the non-protein SH groups in maize leaves of vars. *Kneja 434* (A) and *Kneja 605* (B). Values are means ± SE n = 6. Different letters indicate significant differences assessed by Fisher LSD test ($P < 0.05$) after performing ANOVA multifactor analysis

ment was accompanied by a much stronger oxidative stress in the sensitive plants when compared with the tolerant variety. On the other hand, the tolerant *Kneja 634* control leaves contained increased amounts of non-protein SH groups compared with the susceptible variety. During oxidative stress occurring at chilling temperatures a strong increase in non-protein SH compounds was detected in the leaves of tolerant genotypes of maize [21]. Obviously, Mn tolerance in maize plants can be related to an elevated production of non-protein SH compounds. Thus, the difference in the levels of non-protein SH groups found between sensitive and tolerant maize plants could probably be involved in Mn tolerance. The addition of Si affected non-protein SH content in the leaves of both Mn-treated tolerant and sensitive plants. The addition of Si affected non-protein SH content in the leaves of both Mn-treated tolerant and sensitive plants. Si/Mn-treated plants maintained lower level of non-protein SH groups compared with Mn treatment alone whereas compared with the control the content of non-protein SH was higher. These results support the idea that stimulation of the antioxidant system in plants is the key mechanism of Si-mediated allevation of abiotic stresses [23].

In conclusion, Si improved the growth of Mn-treated maize plants and alleviated Mn toxicity symptoms, thus suggesting that it could play a protective role against Mn damage and thereby increase plant resistance.

ACKNOWLEDGEMENTS

This work was supported by Grant No. B/1524/2006 from the National Science Fund, Ministry of Education and Science, Bulgaria. Project PISA–INI 14/01.09.2005. The authors would like to thank Rumyana Dikova and Emiliya Kotseva for technical assistant.

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