Effect of Si on the distribution of Na in Barley

Bochrnikova E.A.*, Matichenkov V.V., Benes S.*****

**Institute of Physical-Chemical and Biological Problems in Soil Science Russian Academy of Sciences, Pushchino, Moscow region, 142292, Russia E-mail: mswk@rambler.ru*

***Institute Basic Biological Problems Russian Academy of Sciences, Pushchino, Moscow region, 142292, Russia E-mail: Vvmatichenkov@rambler.ru*

****Department of Plan Science, California State University, Fresno, 2415 East San Ramon Ave., M/S AS72, Fresno, CA, USA, sbenes@csufresno.edu*

Abstract

This laboratory investigation with barley was conducted to determine the mechanism of salt toxicity. For the determination of monosilicic, polysilicic acids and Na in apoplast and symplast of roots, stems and leaves, we used a specifically elaborated methodology. The obtained result has shown that there are several mechanisms available to strengthen plants against Na toxicity through improving Si plant nutrition. Soluble Si compounds can block or delay Na transport in apoplast. Monosilicic acid protects chlorophyll molecules against the effect of Na demolition. Soluble Si reduces the active transport of Na into root apoplast. The cells in symplast of barley roots and stems have strong mechanisms for blocking of Na thus preventing sodium toxicity. On the other hand the optimization of Si plant nutrition can also initiate additional penetration of Na into root symplast. The obtained data also demonstrated that the

main reserve of active Si, is locates in barleys leaves. When plants feel stress, this Si- reserve can be rapidly transported to problematic areas. This new methodology gives us the possibility to direct our investigation of plant physiological processes to a new level of knowledge. However, the obtained data has demonstrated the necessity for addition investigations.

Key worlds: salt toxicity, monosilicic acid, polysilicic acid, apoplast, symplast, Na transport

Introduction

Soil salinization is a worldwide problem. The UNEP (United Nations Environment Program) estimates that 20% of the agricultural land and 50% of the cropland in the world is salt-stressed (Flowers and Yeo, 1995). Mismanaged irrigation systems and the resulting salinity to varying degrees are undermining the productivity of at least one third of $230x10^6$ ha of the world's irrigated land (Ramagopal, 1993). It is estimated that irrigated agriculture in the world has increased approximately 300% during the last 35 years (Boyer, 1982). The steady growth of population demand for agricultural products, the need to confront salinity problems is urgent. Also the utilization of more land area for housing and industrial activities forced agriculture onto marginally productive areas which are characterized often by salinity and shortage of water. The increasing plant resistance to salt toxicity today is his top priority.

Last decade numerous investigations have been reported that salt tolerance of cultivated plants could be markedly enhanced by the addition of soluble silicon (Ahmad, 1987; Ahmad et al., 1992; Bradbury and Ahmad, 1990; Matichenkov, Kosobryukhov, 2004; Matichenkov et al., 2006). Silicon (Si) is seconds abundant in the soil after oxygen, which basically represent by various minerals. However this element is a major constituent of many plants as well, but its roles in plant biology have been poorly understood (Liang 1999). Although Si has not been listed among the generally essential elements of higher plants, there have been reports of direct effect of Si supply on plant defense system (Epstein 1999; Gong *et al*. 2006; Liang *et al*. 1996). Several mechanisms of the influence Si on plant defense system were separated (Biel et al., 2008) First mechanism is mechanical via accumulation in epidermal tissue and formation thick epidermal layer, which protect plants against fungi, insect attacks (Ma, Takahashi, 2002). Second is physiological protection where Si increase plant viability thought optimization of root formation process, improvement of photosynthesis process et al (Matichenkov, Kosobrukhov, 2004; Snyder et al., 2006). Third is chemical protection, which realized via chemical reaction between monosilicic acids and pollutants or contaminants in symplast or apoplast of plant tissue

(Matichenkov, Bocharnikova; 2001). This mechanism is supported by high concentration of mono and polysilicic acids in plan sap (Matichenkov et al., 2008). Finally, soluble forms of Si can play role in additional catalytic synthesis of specific and non-specific stress ferments and antioxidants (Biel et al., 2008).

The mechanism of plant defense system against salt toxicity, which reinforcement by Si is poorly understood. Wang and Hang (2007) hypothesize that Si alters the distributions of Na and some trophic ions in alfalfa plants to improve the salt tolerance in salt stress environments. Authors conclude that Si may act to alleviate salt stress in alfalfa by inhibiting Na uptake by roots and affecting in the shoots. Liang et al. (1996) demonstrated that added silicon increased salinity tolerance of barley grown hydroponically. Added silicon enhanced the growth of salt-stressed barley which was found to have improved photosynthetic activity and the ultrastructure of leaf cell organelles (Liang, 1998) and reduced electrolytic leakage of the leaves (Liang et al., 1996). Further studies indicated that silicon enhanced K:Na selectivity ratio (S*k;Na*), which mitigated against the toxic effects of sodium (Liang et. al., 1996).

As well known, selective uptake of mineral ions is associated with the activity of HC-ATPase (Marschner, 1995). One possible mechanism for stimulating effect of Si on KC

uptake by plants under salt stress is, therefore, assumed to be the activation of HC-ATPase in the membranes. Application of Si to salt treated barley with respect to lipid peroxidation and SOD activity in leaves, HC-ATPase activity in roots, and sodium, potassium and calcium accumulation in the shoots and roots. (Liang et al., 1996; Liang, 1998).

Silicon also may act to alleviate salt stress in barley by decreasing the permeability of plasma membranes by helping these structures to maintain their form. Leaf superoxide dismutase and root HC-ATPase activities increased and leaf MDA concentration decreased significantly when the salinised plants were treated with Si. Sodium uptake and transportation into shoots from roots was greatly inhibited by added Si under salt stress conditions, while shoot and root K concentrations in salinised plants were enhanced by added Si (Liang, 1999).

The changing of the cell membrane properties by improving of plant Si nutrition can be also provide the mechanism of protection plant against Na, toxicity (He et al., 2009). However, in this case is not understandable, the presence of the mechanism for fast delivering of Si in to stems of plant (transformation from leaves to stem) and possibility very fast reduction of the toxic effect (Biel et al., 2008).

It was important to investigate the effect of soluble Si on the Na uptake and distribution with using new methodology of plant investigation (Matichenkov et al., 2008). The simple and highly informative methods were elaborated for determination of element or substance content in symplast and apoplast of plant tissue. The main aim of this investigation was to determine the influence of Na and soluble Si on the uptake and distribution of monosilicic, polysilicic acids, sodium in symplast and apoplast of barley roots stems and leaves.

Materials and Methods

The greenhouse experiment was conducted under natural sunlight and photoperiods in California State University Fresno, Department of Plant Science. The temperature was supported by 24/20^{o}C at day/night with supporting of the optimum irrigation during one month. Plastic pots with volume 2 L were filled with 2 kg each of sieved air dried soil that was taken from a cultivated area .

Barley (*Hordium vulgares L*.) variety ( ) was used in this study. 20 seeds were putted to each pot. The irrigation was realized by local water, which contained 26±2 ppm of Si and monosilicic acid and has pH = 7.3, the content of Na was 87±3 ppm of Na. After one month plants were carefully removed from pots, washed in distilled water and replaced to vessels with

distilled water, Si solution, Na solution or combination of Na and Si solution. Distilled water does not have any detectable level of Si or Na. The silicon solution contained 150±3 ppm of Si as monosilicic acid, no detectable level of polysilicic acid and 70±4 ppm of Na. Sodium solution contained no detectable level of any form of soluble Si and 12000±10 ppm of Na. The combined solution contained 150±3 ppm of Si as monosilicic acid, no detectable level of polysilicic acid and 12000±10 ppm of Na.

Twenty five (25) plants, with an average of fresh weight of 2±0.08 g, per plant, were putted into 1 liter volume vessel with solution. Evaporation of the solution was prevented by plastic cover. Plant tissues were sampled after 0, 24, 48 and 96 hours after putting plants into vessels with solutions.

The content of monosilicic acid, polysilicic acids, and Na were determined in the symplast and apoplast of roots stems and leaves by the following methodology.

To determine the contents, tested elements in apoplast fresh specimens were cut into fragments 2.0–2.5 cm in length and 0.25 g in weight, put into a flask containing 50 ml of distilled water and shaken during 24 hours. During 24 hour all apoplast was washed in to solution (Matichenkov et al., 2008). The samples of plant tissues obtained after filtering the remaining solution were homogenized in a mortar and mixed with the new

portion of distilled water (40 ml) again. After that, the suspension was shaken for 60 min. The supernatant was centrifuged during 20 min at 5000 r/m for removing of colloids. The amounts of tested elements again were tested in second solution, which correspond with soluble substances in symplast (Matichenkov et al., 2008). The total Si and Na also was determined in the dried (75 ^{o}C during 4 days) and grounded samples of root, steams and leaves after 96 hours of experiment. The dissolving of plant tissue was realized by Elliot and Snyder (1991) methodology, where NaOH was replaced by KOH. Soluble forms of Si in samples were determined by the following methods, which remove the influence of phosphorus.

Prepare the following reagents:

1. Ammonium molybdate: Dissolve 10 g of [{$(NH_4)_6Mo_7O_{24}$ $4H_2O$] in 470 mL of deionized water and add 30 mL concentrated HCl. Store the solution in a plastic-stored bottle.
2. Reduction solution: Dissolve 20 g of Oxalic acid in 500 mL of deionized water and add 6 g of FeSO4. Dilute 250 mL of concentrated H_2SO_4 (18M) to 250 mL with deionized water. Mix both solutions.

Transfer an aliquot of sample or Si standard solution that contains 2 to 40 μg Si to a 50 mL volumetric flask. Add 10 mL of ammonium molybdate solution and wait 10 min. Add 10 mL of

reduction solution and bring to volume with deionized water and mix well. Measure the absorbance at 660 nm after 3 hours and before 24 hours. Prepare a blank that contains all reagents except the Si solution. This methodology provides the testing only monosilicic acid. Polysilicic acid determination require preliminary de-polymerization, which realized 2 weeks incubation of soluble sample in alkaline condition (add 0.3 ml of 50% NaOH to 20 ml of sample) in the refrigerator at 4 ^{o}C. After that all polysilicic acids will be transferred into monosilicic acid, which give possibility for testing by above described method.

Na was tested by atomic adsorption spectrophotometer (). After testing the content of elements were calculated on the fresh biomass of in plant tissue.

Each treatment was analyzed with at least four replicates, and a standard deviation (S.D.) was calculated. Statistical analysis was performed using the Student's t-test; p≤0:05 and p≤0:001 were considered statistically significant and highly significant, respectively.

Results

In the control plants, the concentration of monosilicic acid in symplast increased in the beginning of experiment in roots and in the end of the experiment in stems and leaves (Table 1).

The application of monosilicic acid in solution resulted in increased of monosilicic acid in root apoplast and symplast and slight increase in symplast of stems and leaves. The dynamic of polysilicic acid in apoplast and symplast in the presence of Si was more complicated (Table 2).

In course of the experiment in root apoplast, the concentration of polysilicic acid decreased and in root symplast it increased. In the control plants, Na concentrations decreased in root and stem apoplast and stayed practically stable in leaf apoplast (Table 3). In the symplast of all tested plant tissues, Na was slowly reduced. In the solution of monosilicic acid, root symplast and apoplast, Na significantly increased (Table 3), whereas the Na in leaf symplast was reduced from 2023 to 984.5 ppm Na. The fact of increasing Na in apoplast and symplast of roots and stems can be explained by Na plant uptake from Si solution since it contained about 70 ppm of Na.

In the presence of high Na concentration in the solution, the accumulation and distribution of monosilicic and polysilicic acids in symplast and apoplast in barley had significantly changed (Table 1 and 2).

Sodium reduced monosilicic acid in plant apoplast and increased monosilicic acid in root, stem, and leaf symplast from

264.9 to 418.5, from 87.2 to 203.3 and from 79.0 to 151.2 ppm Si, correspondingly.

The 96 h-dynamic of polysilicic acid in root and stem apoplast had sinusoid regularity (Table 2). In root symplast, polysilicic acid increased from 1136 to 2640 ppm of Si during the first 2 days and then reduced to 1628 ppm Si.

In stem and leaf symplast, polysilicic acid was staying more constant. The dynamic of Na concentration in plant apoplast under salt toxicity had parabolic form. The increasing Na reached equilibrium in root apoplast in the first 24 h, while in stem apoplast the equilibrium was observed in 48 h of the experiment and in leaf apoplast in 96 h (Table 3). The penetration of additional Na into root and stem symplast was not detected (Table 3). Considering that volume of apoplast is small (literature) the obtained data showed that the main part of Na is accumulated in leaf symplast.

In the solution containing Na + Si, the concentrations of monosilicic and polysilicic acids in root apoplast significantly increased from 171.8 to 565.6 and from 616.9 to 1738 ppm Si, accordingly (Table 1 and 2). The concentrations of monosilicic acid in stem and leaf apoplasts remained stable, while in the corresponding symplasts monosilicic acid was gradually increasing up to the values as those in the variant of Si without

Na. The Na dynamic in apoplast of barley keeping in Si-Na-bearing solution had three parts. In the beginning the content of Na was retained approximately in the same amount, however after 24 hours of the experiment the content of Na in apoplast dramatically increased and then after 48 hours the speed of increasing of Na content was reduced (Table 3). The Na in root symplast has not changed, while the Na in leaf symplast significantly increased (Table 3). The maximum concentration of Na 12606 ppm was detected in leaf symplast in the case of Si+Na solution, it was much higher than that (7962 ppm Na) in the case of Na solution.

Table 1 Dynamic of monosilicic acid in apoplast and symplast in various tissues, of Barley Si ppm.

Solution	Apoplast				Symplast			
	Time of exposure to NaCl, h				Time of exposure to NaCl, h			
	0	24	48	96	0	24	48	96
Roots								
Control	171.8±15.4	138.5±12.4	103.5±6.7	56.2±6.7	264.9±22.3	315.4±34.4	241.1±20.6	222.1±19.3
Si	171.8±15.4	186.5±16.9	174.5±15.4	228.7±23.4	264.9±22.3	294.4±30.2	283.5±22.4	686.3±56.7
Na	171.8±15.4	144.6±15.4	135.2±7	92.0±5.6	264.9±22.3	239.8±28.3	244.5±28.5	418.5±32.6
Na+Si	171.8±15.4	412.4±22.5	352.3±7	565.6±34.8	264.9±22.3	443.4±40.2	408.0±32.4	662.7±38.9
Stems								
Control	69.9±5.8	60.9±6.7	68.1±5.6	30.5±4.5	87.2±4.8	65.2±2.8	99.9±10.4	166.0±15.4
Si	69.9±5.8	70.6±6.8	63.2±4.4	73.6±8.7	87.2±4.8	119.8±4.5	114.4±11.3	195.2±20.3
Na	69.9±5.8	98.5±8.4	100.2±5.8	88.6±4.4	87.2±4.8	155.9±6.6	64.2±4.8	203.3±20.4
Na+Si	69.9±5.8	88.6±4.5	104.7±6.4	91.7±5.4	87.2±4.8	122.3±6.7	119.7±9.2	203.8±23.4
Leaves								
Control	120.4±12.3	55.3±5.5	68.0±4.3	47.3±3.4	79.0±3.5	98.5±5.8	52.2±5.9	123.1±14.3
Si	120.4±12.3	91.9±8.8	91.9±4.4	81.6±6.5	79.0±3.5	63.7±7.6	78.6±4.9	160.0±13.2

Na	120.4±12.3	97.4±3.5	117.3±5.5	96.4±6.5	79.0±3.5	81.1±5.5	104.9±7.6	151.2±20.3
Na+Si	120.4±12.3	80.0±4.4	113.8±6.2	123.1±5.6	79.0±3.5	72.2±7.7	76.3±8.7	204.3±22.4

Table 2 Dynamic of polysilicic acids in apoplast and symplast in various tissues of Barley, Si ppm

Solution	Apoplast				Symplast			
	Time of exposure to NaCl, h				Time of exposure to NaCl, h			
	0	24	48	96	0	24	48	96
Roots								
Control	616.9±48.5	563.1±65.7	956.5±87.6	243.3±32.4	1136±84	2911±213	4062±432	549.0±48.3
Si	616.9±48.5	532.1±54.8	364.2±33.5	334.5±44.3	1136±84	2015±198	2456±233	2804±216.2
Na	616.9±48.5	509.8±40.5	624.5±78.6	506.5±54.5	1136±84	2286±219	2640±244	1628±145.3
Na+Si	616.9±48.5	810.7±78.3	1065.1±105.5	1738±103	1136±84	3706±344	3888±254	3554±319.2
Stems								
Control	34.5±8.2	59.7±3.4	152.3±16.7	70.2±7.48.4	176.1±18.2	237.7±22.3	399.4±34.2	281.5±23.4
Si	34.5±8.2	73.4±6.5	86.2±7.4	26.0±3.1	176.1±18.2	282.8±32.5	330.5±30.3	318.7±39.4
Na	34.5±8.2	61.0±6.5	187.0±12.6	34.9±3.7	176.1±18.2	350.9±36.4	374.1±34.3	280.9±29.9
Na+Si	34.5±8.2	48.8±5.7	46.7±4.3	38.2±3.5	176.1±18.2	457.4±45.3	412.8±443.5	145.8±17.2
Leaves								
Control	46.5±7.4	36.6±3.2	51.4±4.7	30.1±4.2	209.6±21.8	185.1±16.2	178.5±18.2	175.1±15.2
Si	46.5±7.4	41.2±4.4	38.4±8.7	441.7±55.4	209.6±21.8	180.3±18.2	352.2±25.3	379.6±32.2

Na	46.5±7.4	36.4±4.5	33.3±3.5	83.9±9.4	209.6±21.8	144.5±15.2	320.1±23.4	323.9±32.8
Na+Si	46.5±7.4	86.5±7.4	119.3±8.7	51.3±7.6	209.6±21.8	273.0±20.3	310.3±35.4	241.6±21.8

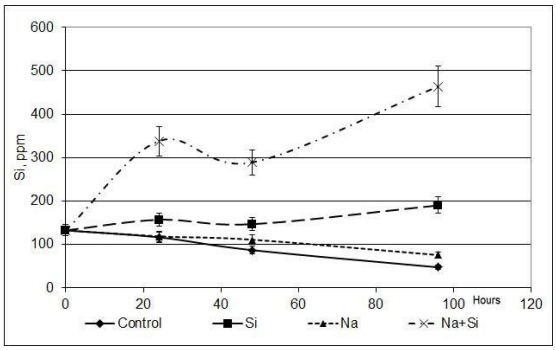
Table 3 Dynamic of Na in apoplast and symplast in various tissues of barley, Na ppm.

Solution	Apoplast				Symplast			
	Time of exposure of NaCl, h				Time of exposure to NaCl, h			
	0	24	48	96	0	24	48	96
Roots								
Control	287.5±29.3	137.6±12.3	171.7±11.5	208.8±27.3	648.6±69.7	334.3±34.5	375.2±44.5	268.6±34.5
Si	287.5±29.3	484.2±35.2	439.0±56.4	820.2±98.4	648.6±69.7	1235±124	1351±152	1335±102
Na	287.5±29.3	10922±995	12017±1045	11592±1020	648.6±69.7	522.4±65.6	507.3±67.4	435.6±34.5
Na+Si	287.5±29.3	4364.9±533	4708±506	8110±843	648.6±69.7	798.9±68.7	778.7±45.6	724.3±89.6
Stems								
Control	289.9±30.5	137.3±12.5	135.2±12.4	171.0±18.3	2456±245	1625±172	2093±109	1727±187
Si	289.9±30.5	193.3±17.5	188.0±17.2	342.9±34.5	2456±245	2503±250	2385±345	2487±205
Na	289.9±30.5	12537±1145	29836±3042	31560±2506	2456±245	2502±234	2885±278	2514±245
Na+Si	289.9±30.5	1758±193	3678.7±345	3762±345	2456±245	3815±389	3856±456	4080±506
Leaves								
Control	61.1±6.7	52.3±4.3	82.6±9.8	67.0±7.6	2023±205	2149.7±205	1550±99	1346±108
Si	61.1±6.7	45.8±3.8	32.4±4.5	53.9±5.6	2023±205	1276±112	1092±108	984.5±87.6

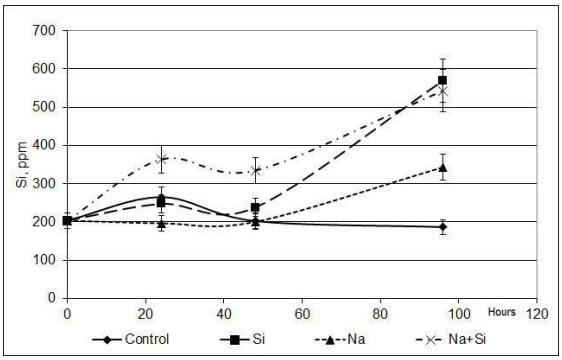
Na	61.1±6.7	17950±1853	30211.1±2500	38663±1280	2023±205	7962±567	7920±544	2228±205
Na+Si	61.1±6.7	1186.1±105.6	2860±176	3450±340	2023±205	2926±345	8944±943	12606±1340

Figure 1 Dynamic of monosilicic acid in plant tissues

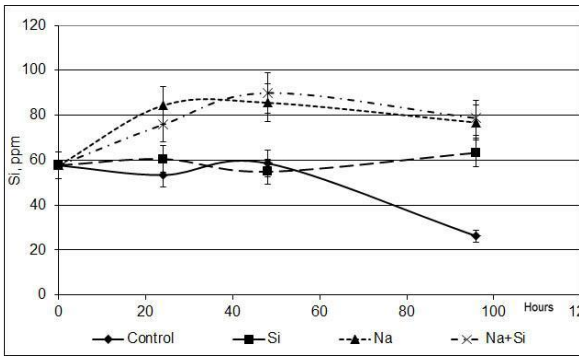
Apoplast of roots



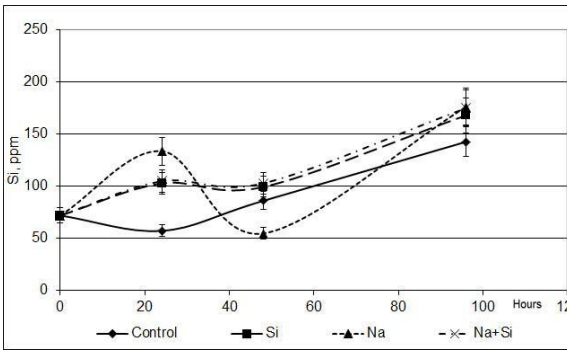
Symplast of roots



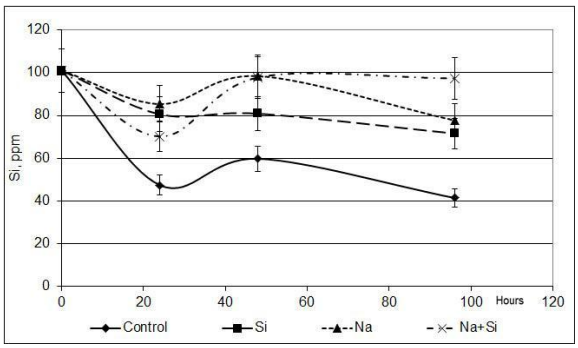
Apoplast of stem



Symplast of stem



Apoplast of leaves



Symplast of leaves

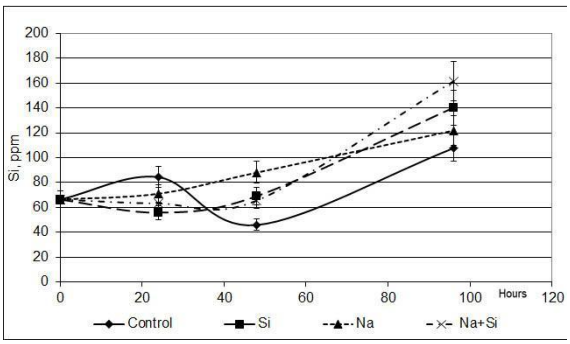
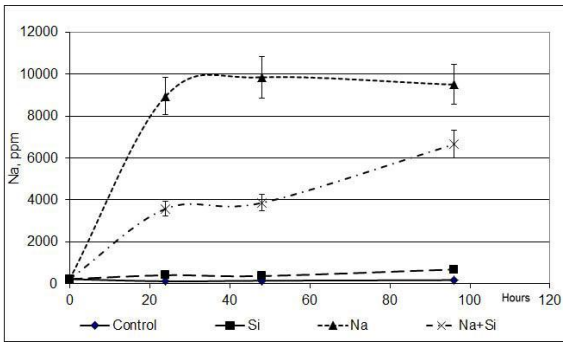
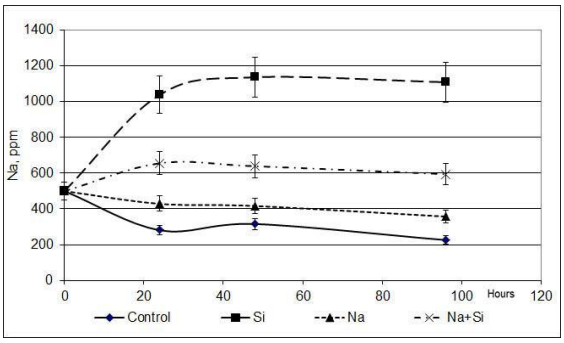


Figure 3 Dynamic of Na in plant tissues

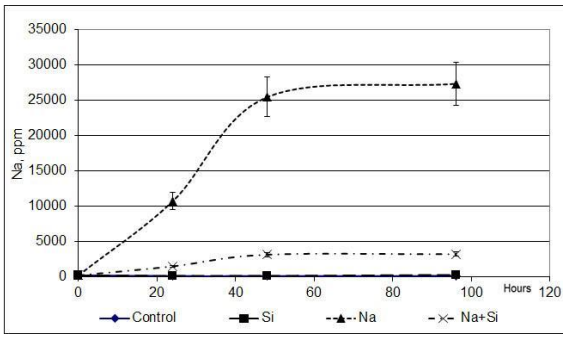
Apoplast of roots



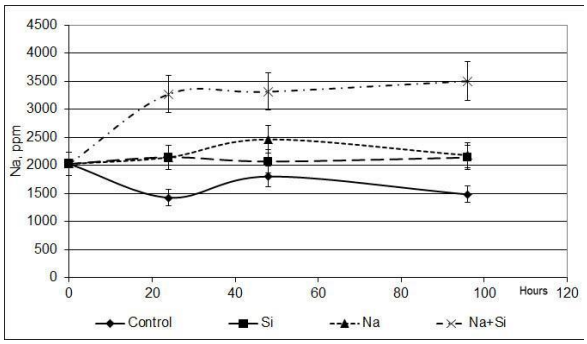
Symplast of roots



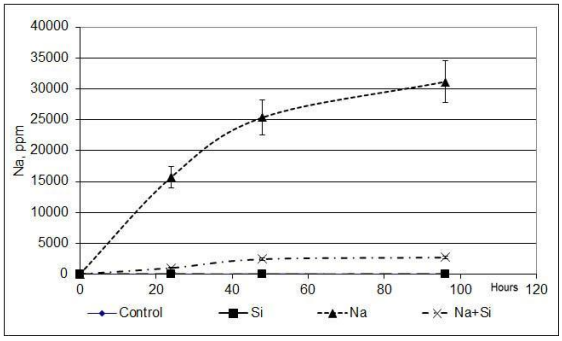
Apoplast of stem



Symplast of stem



Apoplast of leaves



Symplast of leaves

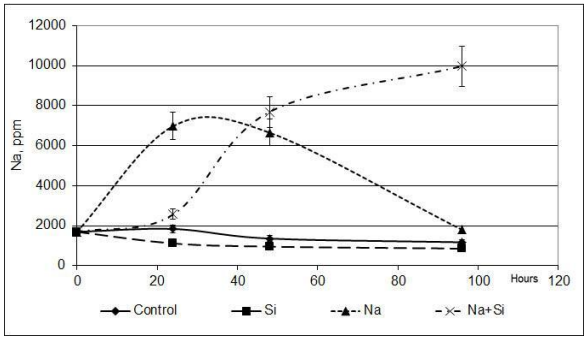
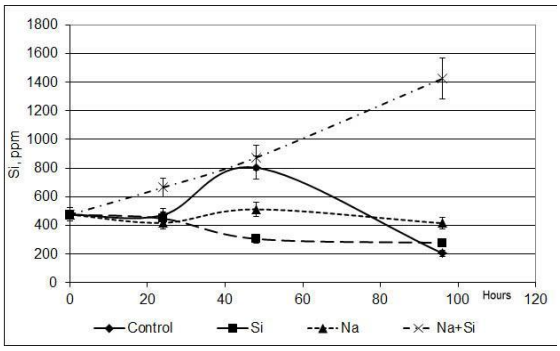
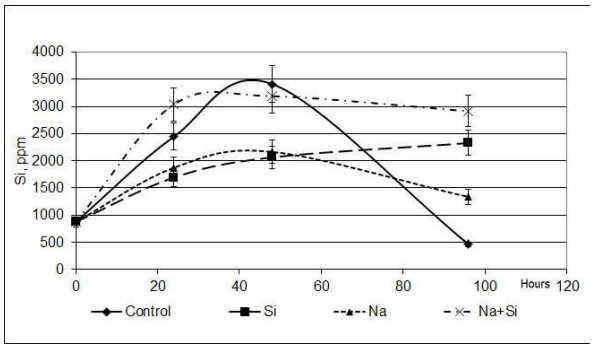


Figure 4 Dynamic of polysilicic acids in plant tissues

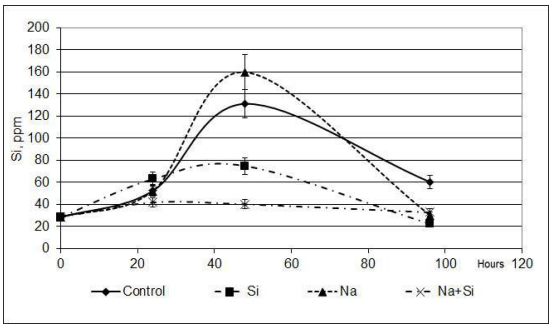
Apoplast of roots



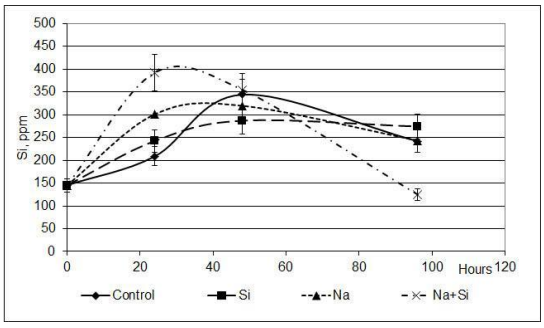
Symplast of roots



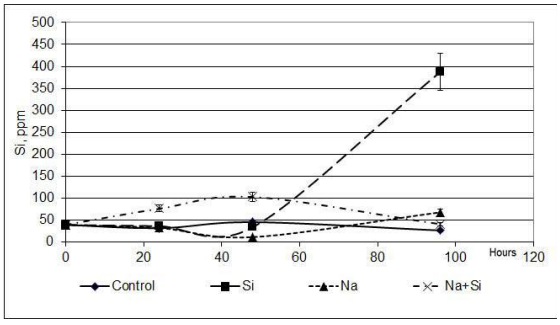
Apoplast of stem



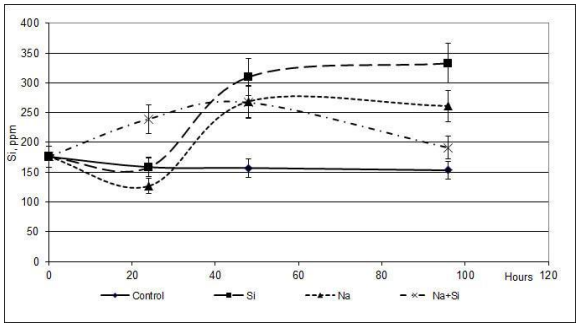
Symplast of stem



Apoplast of leaves



Symplast of leaves



The total plant Si and Na were analyzed after been kept in the solutions for 96 hour (Table 4). As evidenced from the results, total Si increased in all tissues tested in the cases of Si, Na or Na+Si solutions. The maximum total Si was detected in roots and stems from Na+Si solution. In the leaves, the maximum Si was detected for variant of Si-rich solution. Sodium initiated redistribution of Si from leaves to stems and roots.

Total Na in roots was the highest in variant with Si+Na solution (1.23%), while in stems and in leaves the highest level of Na was detected for the variant with Na solution.

Table 4 Total Si in barley before and after been kept in Si, Na and Na + Si solutions, %.

Solution	Before experiment		After experiment	
	Si	Na	Si	Na
Roots				
Control	0.89±0.05	0.038±0.004	0.93±0.08	0.32±0.05
Si			1.31±0.11	0.36±0.07
Na			1.59±0.12	1.11±0.06
Na+Si			2.11±0.13	1.23±0.05
Stems				
Control	0.49±0.09	0.029±0.005	0.43±.09	0.29±0.07
Si			0.89±0.10	0.34±0.06
Na			0.57±0.11	1.92±0.09
Na+Si			0.92±0.08	0.85±0.09
Leaves				
Control	0.72±0.09	0.052±0.006	0.68±.09	0.51±0.06
Si			1.35±0.11	0.64±0.05
Na			0.53±0.08	1.05±0.04
Na+Si			0.78±0.9	0.85±0.05

The results of the tests for monosilicic acid and Na, kept in the solutions after the experiment, are presented in Table 5. In the case of distilled water, Si and Na from plant tissue partly migrated into water solution. In Si-rich solution, the concentration of monosilicic acid decreased from 150 to 122 ppm Si. In Si + Na solution, the concentration of monosilicic acid was reduced to 116 ppm Si. The amount of adsorbed Na from Na solution was significantly higher as compared with adsorbed Na from Si + Na solution.

Table 5.Concentrations of monosilicic acid and Na, in barley, before and after been kept in the solutions, ppm

Solution	Before experiment		After experiment	
	Si	Na	Si	Na
Control	0	0	8.9±1	19±2
Si	150±3	70±4	122±1	59±2
Na	0	12000±10	6.4±1	11835±10
Na+Si	150±3	12000±10	116±1	11870±10

Discussion

Literature reported that optimization of silicon plant nutrition decreased the leaf total Na in some plants (Gong et al., 2006; Zuccurini, 2008). Silicon treatment effects the concentration of Na in plant sap (Gong et al., 2006). Adding silicate, it decreases the mean Na concentration in the xylem sap of leaves from 48 to 67%. The author has suggested

that silicon deposition in exodermises and endodermis reduces sodium uptake through a reduction in apoplastic transport across the root (Gong et al., 2006). Other work also declared the added Si significantly decreased the Na content in the roots (Wang, Han, 2007). Tuna with co-authors (2007) also suggested that sodium transportation into roots and shoots was modestly reduced by added silicate under salt stress conditions for wheat and this mechanism can be recognized as a major factor for reduction salt toxicity by Si fertilization.

Our experiment showed that the reduction of Na transport is influenced by monosilicic acid as well. However, in our experiment additional stress was simulated as a result of replacing barley plants from soil to solution.

It was hypoxia stress, which had the main effect on the roots. According to our previous results on Si behavior in plants under stress, active Si is transported inside the plant to areas mostly exposed to the stress (Biel et al., 2008). In the current experiment, we observed Si transport from leaves to roots (Table 4). The redistribution of Si occurred into symplast for monosilicic acid and into both symplast and apoplast for polysilicic acid.

The dynamic of polysilicic acid concentration in roots and stems had the following regularity. In the beginning of the stress effect, the concentration of polysilicic acid increased rapidly. However, if plant tissue was going to dye the polysilicic acid content in such cell dropped. This regularity was observed in symplast of roots in control and under salt toxicity and hypoxia. For example in roots under salt stress and hypoxia, the concentration of polysilicic acid increased more than 130% and then again it was reduced.

In the presence of Si in the solution, the concentration of monosilicic acid significantly increased only in symplast of all tissues, while the increase in monosilicic acid in apoplast was observed only in roots. The redistribution of Si from leaves to roots was fixed on the results of total Si in plant tissue after the experiment. Without stress higher accumulation of adsorbed Si was observed in leaves (Table 4). We suggest that leaves are places where barley can accumulate Si, which can be transport to tissue under stress attack. Any stress was found to initiate redistribution of Si inside of plants with Si transported to problematic places.

We suggest that polysilicic acid is the main form in which Si is transported in plant tissue. By this means the decreasing polysilicic acid in apoplast of barley roots in the

presence of added Si can be explained. The new-formed polysilicic acid from roots immediately transported to leaves and stems as well as partly polysilicic acid penetrated to root symplast for protection of roots against hypoxia stress.

The results of analyzing solutions after the experiment have shown that some Na was desorbed from plants (Table 5). The symplast of barley's root and stems has very strong protection against penetration of Na from apoplast. At the same time the content of Na in root symplast remarkably increased as a result of staying in Si-rich solution with low level of Na. By this means, the transport of Na in this symplastic cells can be realized only by active transport with signal system, which recognized not only critical amount of Na in apoplast and can completely block penetration of Na into symplast, but also can accelerate Na transport into symplast, if present additional content of plant available Si. Probably, Si participates in the regulation of the active transport of nutrients, including sodium. The complex formation between monosilicic acid and sodium can play an important role in this process, because the size of complex molecules will be much bigger that size of sodium anion. On the other hand, the formation of such complexes can explain blocking of Na transport, but can't clarify the acceleration of

the Na movement into symplast, which was observed in our experiment with monosilicic acid. The reduction of sodium in leaf symplast in the finish of the experiment can be described by reduction of moisture in symplast, which recognized the death of cell.

Such aggressive stress as sodium initiated the increasing of both monosilicic and polysilicic acids content in the root apoplast more intensively than hypoxia stress. This data is related with other publications, which demonstrate that increasing Si in plant tissue under stress is in direct proportion to intensity of stress (Biel et al., 2008; Matichenkov, 2008).

Our data also showed that enhancing silicon uptake by plants exposed to stress (Table 5). Probably, this is related with plant signal system, which controls the active adsorption of Si by roots. The passive transport can't be realized, because the concentration of monosilicic acid in root apoplast and symplast much higher than in external solution. The data present in Table 1 shows that the concentration of monosilicic acid in apoplast and symplast are higher than their concentration in nutrient solution. However, real concentrations of monosilicic acid in apoplast could be several times higher because in our calculations we used

just total volume of plant sap, which was determined by plant moisture. The real volume of apoplast is much smaller than total volume of tissue (Literat). By this means, the transport of monosilicic acid to roots occurs against concentration gradient.

In the solution rich in Si and Na, the Na transport in barley apoplast was changed, compare with Na movement thought plant tissue from Na-rich solution. The obtained data clearly shows that under improvement of Si plant nutrition Na transport was suppressed (Table 3). First, Si reduced plant absorbed Na (Table 5). Secondary, the reduction of Na transport was observed in root apoplast, then in stems and finally in leaves. The reduction of Na in root, stem and leaf apoplast was realized on 31%, 88.1% and 92%, correspondingly. We suggest that Si realize successive blocking of Na transport via apoplast transport system. The high level of the reduction Na content in apoplast (for example in leaf apoplast was changed from 38663 Na ppm to 3450 Na ppm (11.2 times!). The delay of Na penetration to leaf symplast clearly demonstrated by obtained data as well. Summarizing of the data obtained we suggest that this is major mechanism for reduction of Na toxicity by active forms of Si. However, our data indicates the availability of the

second mechanism of the Si protection against salt toxicity. The symplast of plant treated by Si-Na-bearing solution contained much more Na than symplast of barley under Na-bearing solution; however these cells had more viability, compared with symplast under treatment by Na-bearing solution. Additional Si protects plant cell against sodium destruction inside the cell. Probably this mechanism related with possibility to reinforce chlorophyll molecules by monosilicic acid and protect them against Na demolition. This hypothesis was suggested several years ago (Matichenkov et al., 2005; Zuccarini, 2008).

Conclusions

The obtained data give possibility to suggest the following conclusions. Several mechanisms are available for the prevention of sodium toxicity by soluble silicon compounds. The blocking or reduction of Na active transport in apoplast can be realized in roots, stems and leaves. Probably this mechanism is realized thought complex formation of monosilicic or polysilicic acids with sodium. Monosilicic acid also increased the resistance of chlorophyll molecules against their demolition by sodium, in the result the symplast cell is kept viable under high concentration of Na in sap.

Soluble Si also can reduce the adsorption of Na from solution. Probably all these mechanisms are at work simultaneously. Root and stem cells in symplast have the possibility to regulate the penetration of Na. If in apoplast media the concentration of Na increased dramatically, the symplastic gates for element penetration can stop Na penetration. On the other hand the optimization of Si nutrition together with small increase of Na concentration in solution and in apoplast can result in increased Na in symplast or roots. This means that symplast cell of roots and stem of barley has perfect protection against Na toxicity. The active transport of silicon in plant, is probably realized by polysilicic acid, which is primarily formed in the root zone. The accumulation of Si in plant without stress or low stress is realized in leaves of barley. Under stress this source of Si can be re-distributed to problematic places. The concentration of polysilicic acid rapidly increases in problematic places and if stress results in cell death, the content of polysilicic acid dramatically drops.

New methodological approaches provide the next level of information about processes and mechanisms, which take place in plant tissue. However additional experiments and investigation are necessary in order to verify the basic

mechanisms of reduction of salt toxicity by silicon fertilizers. However the obtained data showed how important is the use of this type of Si- fertilizers for salt-affected soils.

Acknowledgement

This project was supported by Fulbright Program.

Activated Silicon: ZumSil, supplied by Terra Tech Intl.

Literature review

1. Ahmad R 1987 Development of Silicon Technology for Saline Agriculture. Research report, Pakistan Atomic Energy Commission, Botany Department, Karachi University.
2. Ahmad R, Zaheer S, Ismail S. 1992. Role of silicon in salt tolerance of wheat (*Triticum aestivum L*.). Plant Sci 85:43-50.
3. Biel K.Y.**,** Matichenkov V.V., Fomina I.R. Protective role of silicon in living systems // *In*: Functional Foods for Chronic Diseases. Advances in the Development of Functional Foods, DM Martirosyan (Ed.), Copyright © by D&A Inc., Richardson, Texas, USA, 2008. V. 3. P. 208-231.
4. Boyer, J. S. (1982). Plant productivity and environment. Science, 218: 443-448.
5. Bradbury M, Ahmad R 1990: The effect of silicon on the growth of *Prosopis juliflora* growing in saline soil. Plant Soil, 125, 71–74.
6. Elliot CL, Shyder GH. 1991. Autoclave-induced digestion for the colorimetric determination if silicon in rice straw. J Agric Food Chem 39:1118-1119.

7. Epstein, E., 1999. Silicon. Annu. Rev. Plant Physiol. Plant Mol. Biol. 50, 641–644.
8. Flowers, T.J. and A.R. Yeo, 1995. Breeding for salinity resistance in crop plants. Aust. J. Plant Physiol., 22: 875-884.
9. Gong HJ, Randall DP, Flowers TJ 2006: Silicon deposition in the root reduces sodium uptake in rice (Oryza sativa L.) seedlings by reducing by pass flow. Plant, Cell Environ., 29, 1970–1979.
10. He Y., Xiao H., Wang H., Chen Y., Yu M. 2009. Effect of silicon on chilling-induced changes of solutes, antioxidants, and membrane stability in seashore paspalum turfgass. Acts Physiol Plant DOI 10.1007/s11738-009-0425-x
11. Liang Y C, Shen Q R, Shen Z G and Ma T S 1996 Effects of silicon on salinity tolerance of two barley cultivars. J. Plant Nutr. 19, 173–183
12. Liang Y C 1998 Effects of Si on leaf ultrastructure, chlorophyll content and photosynthetic activity in barley under salt stress. Pedosphere 8, 289–296.
13. Liang Y. 1999. Effects of silicon on enzyme activity and sodium, potassium and calcium concentration in barley under salt stress. Plant Soil 209:217-224.
14. Ma JF, Takahashi E. 2002. Soil, fertilizer, and plant silicon research in Japan. Elsevier, The Netherlands.
15. Marschner H 1995 Part I. Nutritional physiology. *In* Mineral Nutrition of Higher Plants. Ed. H Marschner. pp 18–30, 313–63. Academic Press Limited, London. Second edition.
16. Matichenkov V.V., Bocharnikova E.A., Kosobryukhov A.A., Biel K.Y. 2008. Mobile forms of silicon in plans. Diklady Biological Sci. 418:39-40.
17. Matichenkov V.V., Kosobryukhov A.A., Shabnova N.I., Bochernikova E.A. 2005. Reaction of plant on

silicon fertilizers under soil salinity. Agrochemistry, 10:59-63.
18. Matichenkov VV, Kosobryukhov AA. 2004. Si effect on the plant resistance to salt toxicity. In *Proc* 13th Intern Soil Conservation Organization Conference, July 4–9, 2004, Brisbane, Australia., pp 287-294.
19. Matichenkov V.V. 2008. Role of mobile compounds of silicon in plants and soil-plants system. Authoreff. Doctoral Diss. Institute Basic Biological Problems RAS, Pushchino, 34 p.
20. Matichenkov VV, Bocharnikova EA. 2001. The relationship between Si and soil physical and chemical properties. In, Silicon in Agriculture. Studies in Plant Science. Datnoff L, Snyder G, Korndorfer G (ed), pp. 209-219. Elsevier, Amsterdam.
21. Ramagopal, S. (1993). Advances in understanding the molecular biology of drought and salinity resistance inplants —the first decade. Adv Pl Biotech Biochem (Eds. Lodha, M.L.; Mehta, S.L.; Ramagopal, S. and Srivastava, G.P.) Indian Soc Agril Biochemists, Kanpur, India, pp. 39-48.
22. Snyder GH, Matichenkov VV, Datnoff LE. 2006. Silicon In: Handbook of Plant Nutrition, Massachusetts University, p.551–568.
23. Tuna A. L., Kaya C., Higgs D., Murillo-Amador B., Aydemir S., Girgin A.R. 2007 Silicon improves salt tolerance in wheat plants. Environmental and Experimental Botany, 62: 10-16.
24. Wang X.Z., Hang J.G. 2007. Effects of NaCl and silicon on ion distribution in the roots, shoots and leaves of two alfalfa cultivars with different salt tolerance. Soil Sci. and Plant Nutr. 53: 278-285.
25. Zuccarini P. (2008) Effects of silicon on photosynthesis, water relations and nutrient uptake of

Phaseolus vulgaris under NaCl stress. Biol Plantarum, 52(1): 157-160.