



## Interactive effects of silicon and NaCl on some physiological and biochemical parameters in *Borago officinalis* L.

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### Abstract

In this research we investigated the role of Si in improving salt tolerance in the medicinal plant *Borago officinalis* L. *Borago* was pretreated with 0, 0.5 and 1.5 mM Si (as sodium silicate, Na<sub>2</sub>(SiO<sub>3</sub>)<sub>2</sub>) and then treated with four levels of NaCl, namely, 0, 60, 90 and 120 mM NaCl. Then the effects of silicon and NaCl were observed on some physiological and biochemical parameters such as lipid peroxidation malonaldehyde (MDA) and other aldehydes, proline, protein and carbohydrate contents. NaCl significantly increased MDA, other aldehydes and proline contents. Addition of 0.5 mM Si decreased the level of reducing sugars in salt-stressed plants in the leaves. But in plants pretreated with Si and then exposed to NaCl, MDA, other aldehydes contents and carbohydrate in the roots decreased significantly. Protein content on the other hand, increased significantly (P<0.05). Results showed that pretreatment with Si can alleviate NaCl stress in the plants under study.

**Keywords:** *Borago officinalis*; silicon; salinity

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### Introduction

Silicon is the most abundant element in the soil for most of world's plants (Epstein, 1994). This element is also one of the most abundant mineral elements in plant tissues (Zhu et al, 2004). Although silicon is not considered an essential nutrient for higher plants, its beneficial effects on plant growth and development have been proven by numerous studies concerned with its role in plant nutrition (Marschner, 1995;

Epstein, 1999). Improvement of plant growth by addition of Si is beneficial for increased tolerance of bean tissues to high manganese concentration (Horst and Marschner, 1978). In another research it has been reported that Si enhances the salt tolerance of mesquite and wheat (Ahmad et al. 1992).

Plant tolerance to diseases, drought, metal toxicities, and salt stress was also reported to increase with Si (Epstein, 1999; Richmond and Sussmsn, 2003; Ma, 2004). Some studies showed that in some plants Si treatment was associated with antioxidant defense abilities (Epstein, 1999; Richmond and Sussmsn, 2003; Ma, 2004).

Salinity is the major stress factor, which limits crop plants cultivation. Approximately one-

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third of the world land surface is arid and semi-arid, of which one half is affected by salinity (Liang et al., 1996). Salinity is also an environmental factor that has a major effect on plant quantity and quality (Zhu, 2002). Many attempts have been made to alleviate the effects of salinity on plant production in saline soils. It was shown that the exogenous application of Si enhanced the growth of some higher plants and increased salinity tolerance in them (Liang et al., 2007).

A medicinal plant, borage has thick leaves that taste of cucumber and its seeds contain crude protein and high levels of oil with pharmaceutical uses. Gamalynolek acid found in borage seed oil is a generally rare fatty acid in plants which is used as food supplements and drug for the treatment of many diseases including heart disease, diabetes and arthritis (El-Hafid et al., 2002). In this research, we studied the effects of different Si levels on reducing sugars, proline, malondialdehyde, other aldehydes and protein contents of *Borago officinalis* L. under different concentrations of NaCl stress.

Table 1  
12 treatments on nutrient solution (in *Borago*)

No	Name	Treatments
1	S <sub>0</sub> N <sub>0</sub>	contral
2	S <sub>0</sub> N <sub>60</sub>	With 60 mM NaCl
3	S <sub>0</sub> N <sub>90</sub>	With 90 mM NaCl
4	S <sub>0</sub> N <sub>120</sub>	With 120 mM NaCl
5	S <sub>0.5</sub> N <sub>0</sub>	With 0.5 mM Si
6	S <sub>0.5</sub> N <sub>60</sub>	With 0.5 mM Si and 60 mM NaCl
7	S <sub>0.5</sub> N <sub>90</sub>	With 0.5 mM Si and 90 mM NaCl
8	S <sub>0.5</sub> N <sub>120</sub>	With 0.5 mM Si and 120 mM NaCl
9	S <sub>1.5</sub> N <sub>0</sub>	With 1.5 mM Si
10	S <sub>1.5</sub> N <sub>60</sub>	With 1.5 mM Si and 60 mM NaCl
11	S <sub>1.5</sub> N <sub>90</sub>	With 1.5 mM Si and 90 mM NaCl
12	S <sub>1.5</sub> N <sub>12</sub>	With 1.5 mM Si and 120 mM NaCl

## Materials and Methods

### Plant materials

The seeds of *Borago officinalis* were obtained from the Isfahan Agriculture Research Institute (Isfahan, Iran). After being sterilized with 5% sodium hypochloride for 5 min to prevent fungal attack and rinsed with sterile water, the

seeds were allowed to germinate on filter paper saturated with sterile distilled water. Selected seedlings of equal size and vigor were transferred to plastic pots, 15 cm in diameter, containing vermiculite (perlite) in a greenhouse. Seven seedlings were sown in each pot. The total number of pots was 120 (6 pots per treatment). The pots were incubated in a greenhouse with natural light at 18-24 °C and the relative humidity (RH) of 40%. The seedlings were irrigated with water and Hoagland's stock solution alternatively.

### Silicon treatments

Three weeks after sowing, plants were pretreated with silicon for 3 weeks. In the nutrient solutions, 0, 0.5 and 1.5 mM silicon was supplied as sodium silicate (Na<sub>2</sub>(SiO<sub>2</sub>)<sub>3</sub>).

### Salinity treatments

In the nutrient solutions, NaCl was supplied at 0, 60, 90 and 120 mM NaCl (Table 1). Plants were treated with NaCl for one month after pretreatment with silicon.

### Malondialdehyde (MDA) and other aldehydes

A decomposition product of poly unsaturated fatty acids was utilized as a biomarker for lipid peroxidation (Mittler, 2002). The level of lipid peroxidation was quantified by measuring the amount of malondialdehyde (MDA), which is determined by thiobarbituric acid (TBA) reaction as described by Heath and Packer (1969) in  $\lambda=532$  nm. Other aldehydes such as Proponal, Botanal, Hexanal and Heptanal contents were measured according to Meirs, et al. (1992). This method is similar to MDA analysis but  $\lambda$  equals 455 nm.

### Free proline content

Proline was extracted and its concentration determined by the method of Bates et al. (1973). Leaf tissues were homogenized in 3 % sulfosalicylic acid and the homogenate was centrifuged at 3,000×g for 20 min. The supernatant was treated with acetic

acid and ninhydrin, boiled for 1 h, and then the absorbance was determined at 520 nm. L-Proline (Sigma) was used for a standard curve.

### Free protein content

The protein concentration in tissue extracts was measured by the Lowry (1951) method, using bovine serum albumin for drawing standard curve.

### Reducing sugar

Reducing sugar was measured according to the method suggested by Somogy (1952). Leaf tissues were homogenized in distilled water. In this method we used  $\text{CuSO}_4$  and Phospho molibdic acid, and then the absorbance was determined at 600 nm. Glucose (Sigma) was used for a standard curve.

### Statistical Analysis

The experiment was a completely randomized design consisting of all treatments and three replication. Difference between the means were compared through Duncan's multiple range test ( $p < 0.05$ ) using the "MSTAT-C" computer program.

## Results

### Interactive effects of silicon and salinity on lipid peroxidation of *Borago*

The results indicated that sodium chloride treatment led to a significant increase in the content of MDA and other aldehydes, and the

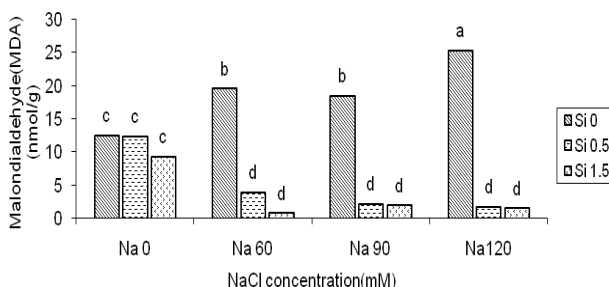


Fig. I. The effects of silicon pretreatment on the changes of the malondialdehyde content (MDA) in the leaves of *Borago* under different NaCl levels. Values with different letters are significantly different at  $p < 0.05$  based on Duncan test.

silica treatments could significantly decrease these metabolites (Figs. I and II).

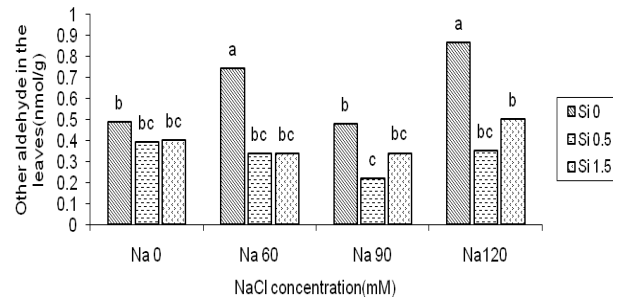


Fig. II. The effects of silicon pretreatment on the other aldehydes contents in the leaves of *Borago* under different NaCl levels. Values with different letters are significantly different at  $p < 0.05$  based on Duncan test.

### Interactive effects of silicon and salinity on proline content in the leaves and roots of *Borago*

The results indicated that high concentration of sodium chloride led to a significant increase in proline content of shoots and root. In the combined treatments of silica and sodium chloride, silica caused a significant decrease in the level of proline in root.

Furthermore, in the combined treatments of silica and sodium chloride at salinity level of 60 mM and concentration of 0.5 mM, sodium silicate led to a significant decrease in the amount of proline in aerial parts, and concentration of 1.5 mM led to marked increase in this parameter. The salinity of 90 mM and concentration of 0.5 mM sodium silicate led to a significant increase in the amount of proline in aerial parts, and concentration of 1.5 mM sodium silicate led to marked decrease in this parameter. In 120 mM NaCl, concentration of 0.5 mM sodium silicate led to a significant decrease in the proline content in aerial parts (Figs. III and IV).

### Interactive effects of silicon and salinity on protein content of *Borago*

The results revealed that sodium chloride treatments led to a significant decrease in the amount of protein content. In combined treatment of silica and sodium chloride, silica caused a significant increase in the amount of protein. (Fig. V)

### Interactive effects of silicon and salinity on reducing sugar in the leaves and roots of *Borago*

The results showed that sodium chloride treatments in concentration of 60 and 90 mM led to a significant decrease in the amount of reducing sugar in aerial parts. In the combined silica and sodium chloride treatments the salinity level of 60 and 90 mM and concentration of 0.5 mM sodium silicate led to a significant decrease in the amount of reducing sugar in aerial parts and concentration of 1.5 mM sodium silicate led to a marked increase in this parameter. Furthermore, in combined silica and sodium chloride treatments, silica led to a significant decrease in the amount of reducing sugar in root especially in 0.5 mM (Figs. VI and VII).

### Discussion

Considering the obtained results, increment of the amount of MDA and other aldehydes and decrement of the amount of protein under salinity indicate that plants in these circumstances would be under the influence of stress and the firmness and action of membrane would face a problem.

The results showed that pretreatment of plants with silica followed by salinity treatment led to a significant decrease in the amount of MDA and other aldehydes which in turn leads to the increment of plant tolerance and amplification of resistance mechanism towards oxidative stress due to sodium chloride. These results are in accordance with those of Liang (1999) and Al-aghabary et al. (2004).

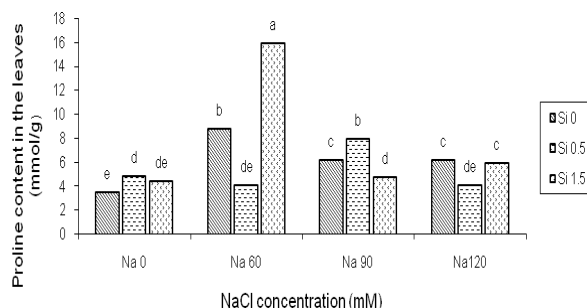


Fig. III. The effects of silicon pretreatment on the proline content in the leaves of *Borago* under different NaCl levels. Values with different letters are significantly different at  $p < 0.05$  based on Duncan test.

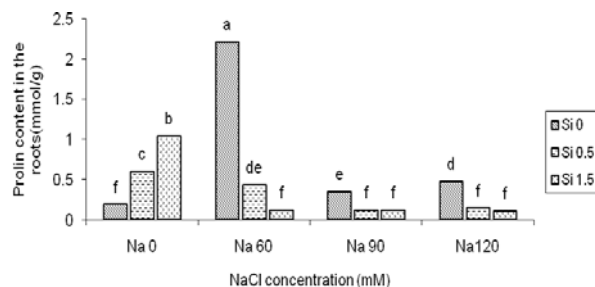


Figure IV. The effects of silicon pretreatment on the proline content in the roots of *Borago* under different NaCl levels. Values with different letters are significantly different at  $p < 0.05$  based on Duncan test.

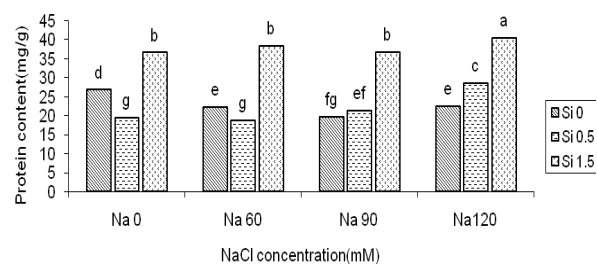


Fig. V. The effects of silicon pretreatment on the protein content in the leaves of *Borago* under different NaCl levels. Values with different letters are significantly different at  $p < 0.05$  based on Duncan test.

In plants which were pretreated with 0.5 mM silica, and then treated with NaCl, the amount of protein significantly increased. This is in accordance with the findings of Liang (1998) and Al-aghabary et al. (2004). Probably, silica has stimulated mRNA synthesis and increased the amount of protein. Silica also caused a decrease in protein destruction as a result of stress due to sodium chloride.

Proline which is a source of osmolyte (carbon and nitrogen) (Hare and Cress 1997), plays a critical role in plant resistance against biotic and abiotic stress. Some researchers showed that proline has a role for scavenging ROS during oxidative stress (Kavi Kishor et al., 2005; Verbruggen et al., 2008). In this research conducted on *Borago*, the amount of proline and reducing sugars in the roots increased under salinity stress and this might be because of the osmotic adjustment mechanism in the plant. Proline and reducing sugars increase as compatible osmolytes in plants would enhance the plant resistance to dehydration and the growth of plant under saline conditions. It looks as if reducing sugar in the roots is related to more salt stress. Moreover, the reason for

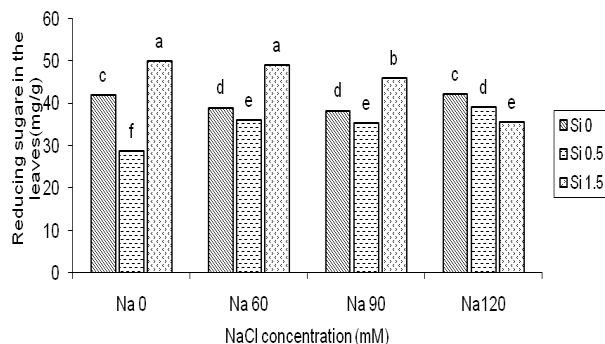


Fig. VI. The effects of silicon pretreatment on the reducing sugar in the leaves of *Borago* under different NaCl levels. Values with different letters are significantly different at  $p < 0.05$  based on Duncan test.

reducing sugar in aerial parts can be the decrement of photosynthesis.

From these results we conclude that silicon increased plant resistance against salinity because MDA content reduced in plants pretreated with silicon and treated with NaCl.

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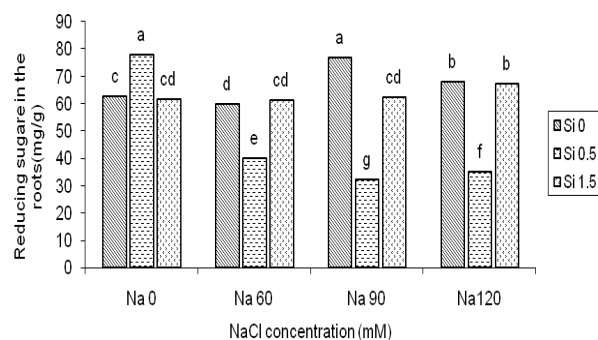


Fig. VII. The effects of silicon pretreatment on the reducing sugar in roots of *Borago* under different NaCl levels. Values with different letters are significantly different at  $p < 0.05$  based on Duncan test.

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