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Effect of Silicon on Growth and Physiological Parameters in Fenugreek (*Trigonella foenum-graceum* L.) Under Salt Stress

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ABSTRACT: A green house experiment was conducted to investigate the effects of silicon application on *Trigonella foenum- graceum* L. under salt stress. The experiment was a 4×2 factorial arrangement with four levels of NaCl in the irrigation water, 0, 60, 120 and 180 mM and two levels of silicon (Si), 0 and 1/5 mM as Na_2sio_3. The result showed that the plants grown at 180 Mm NaCl produced less dry matter and chlorophyll content than those without NaCl. Supplementary Si at 1/5 mM ameliorated the negative effects of salinity on plant dry matter and chlorophyll content. Membrane permeability in leaves increased with addition of NaCl and these increases were decreased with Si treatment. The results indicated that the silicon increased the tolerance to salt stress, in which the leaf relative water content, chlorophylls a and b were maintenanted in higher levels, if compared with stress plants.

Keywords: Salinity stress, Silicon*, Trigonella foenum- graceum* L.

INTRODUCTION

Silicon (Si) is the second most abundant element on the surface of the earth, yet its role in plant biology has been poorly understood and attempts to associate Si with metabolic or physiological activities have been in conclusive (Lewin & Reimann, 1969; Epstein, 1999).

Although Si has not been listed among the generally essential elements of higher plants, it has been demonstrated to be beneficial for the growth of plants, particularly gramineous plants and to alleviate both biotic and abiotic stresses in plants (Okuda and Takahashi, 1965; Epstein, 1994; Liang et al., 1994). Salinity can be minimised with reclamation, water and drainage, but the cost of engineering and management is very high for overcoming the negative of salinity on the palnt growth and yield could be to attempt to supplement. An alternative strategy Si where irrigation water is known to be or may become saline. Silicon improved photosynthetic activity and the ultra structure of leaf cells in barley (liang, 1998), reduced electrolyte leakage in the leaves and enhancing the plant growth at high salinity (liang et al., 1996). Satisfactory result of silicon application against NaCl stress have been shown in rice (Matoh et al., 1986), wheat (Ahmad et al., 1992), *Prospis jaliflora* (Bradbury and Ahmad 1990) and barley in hydroponics (liang et al., 1996, liang, 1998, 1999, liang & Ding, 2002) .*Trigonella foenum- graceum* L. is a large genus with close to 135 species belonging to the family leguminosae (fabaceae). Most of the species are distributed in the dry regions. The objective of this work was to investigate the effectiveness of silicon in mitigating the adverse effects of salinity and to investigate possible mechanisms of silicon enhancement of salt tolerance in Fenugreek.

MATERIAL AND METHODS

Plant culture and treatments

A pot experiment was conducted in controlled environment conditions at the department of Horticulture, Ferdowsi university of Mashhad, Iran. The plant remained in glasshouse environment under natural conditions day/night (air temperature minimum/maximum and relative humidity of 20/32°C and 65%, respectively). The photoperiod medium was of 12h of light. *Trigonella foenum-graceum* L. seeds were surface sterilized with 10% sodium hypochlorite sterile solution and then washed several times with sterile distilled water. Five Seeds pot $^{-1}$ were sowed in 48, 10cm diameter pots containing a 1: 1:1 mixture of sand, peat and perlite on 1March 2008. After the germination were kept 1 plant pot $^{-1}$. For the first 20 days following leaf emergence all of the plants were irrigated daily with tap water. The treatment of plants with NaCl and Sio_2 (as the soluble sodium silicate) began 20 days from leaf emergence. The plants were arranged in randomised 4×2 factorial with 10 replications for each treatment, the plants was irrigated daily with one of the following solutions: control: tap water, control+ 1/5mM Sio_2, 60 mM NaCl+ 1/5 mM Sio_2, 120 mM NaCl, 120mM NaCl+1/5 mM Sio_2, 180mM NaCl, 180mM NaCl+ 1/5Nm Sio_2. Excess solution was allowed to drain from the plants through drainage holes in the base of the pots. A commercially available fertilizer containing N, P and k (10: 10: 27) with trace quantities of Mg, Fe and Mn was added to the pots every week for the duration of the experiment.

Chlorophyll concentration

One plant per replicate (randomly chosen) was used for chlorophyll determination. One gram of fresh leaf material taken from the youngest fully expanded leaf was extracted with 90% acetone and read using a UV/vis spectrophotometer at 663,645 and 750 nm wavelengths. Absorbance at 750 nm was subtracted from absorbance at the other two wavelengths to correct for any turbidity in solution before chlorophyll concentration were calculated using the formulae below from:

Chl.a (mg ml^{-1}) = 15.65(A_{666}) - 7.340 (A_{653}),

Ch.b (mg ml^{-1}) = 27.05(A_{653}) – 11.21(A_{666})

Where A_{666} and A_{653} represent absorbance values read at 663 and 645 nm wavelengths, respectively.

Electrolyte leakage

Electrolyte leakage was used to assess membrane permeability. This procedure was based on Lutts et al (1996). Electrolyte leakage was measured using an electrical conductivity meter. Leaf samples of one randomly chosen plant per replicate were taken from the youngest fully expanded leaf and cut into 1cm segments. Leaf samples were then placed in individual stopper vials containing 10 mL of distilled water after three washes with distilled water to remove surface contamination. These samples were incubated at room temperature (25 ºC) on a shaker (100rpm) for 24 h. Electrical conductivity (EC) of bathing solution (EC_1) was read after incubation. The same samples were then placed in an autoclave at 120 ºC for 20 min and the second reading (EC_2) was determined after cooling solution to room temperature. The Electrolyte leakage was calculated as EC_1/ EC_2 and expressed as percent.

Relative water content (RWC)

Four samples (replications) are taken from a single treatment. Top-most fully expanded leaves are sampled, unless interested in profiling leaves on the plant. Each sample is placed in a pre-weighed airtight (possibly also oven proof) vial. Samples should reach the lab as soon as possible. In the Lab vials are weighed to obtain fresh leaf sample weight (FW), after which the sample is immediately hydrated to full turgidity for 4h under normal room light and temperature. Samples 1 and 2 above are dehydrated by floating on deionized water in a closed Petri dish. Sample 3 above receives water into the vial to a level of 1-2cm after which the vial is capped. After 4h samples are taken out of water and are well dried of any surface moisture quickly and lightly with filter paper and immediately weighed to obtain fully turgid weight (TW). Samples are then oven dried at 80℃ for 24h and weighed (after being cooled down in a desiccator) to determine dry weight (DW). All weighing is donning to the nearest mg. (Barr and Weatherly, 1962).

Calculation: RWC (%) = (FW – DW/ TW – DW) × 100

Dry matter determination

Three randomly chosen plants per replicate were divided into shoots and roots and fruit and washed in detergent solution to remove and dust on leaf surfaces and then dried at 70℃ for 48h to constant weight.

Data analysis

The data were analyzed using a Stat view ANOVA program. Statistically different groups were determined by Duncan̉ s test (P<0.01).The statistical analysis were carried out with the software MSTATC Institute.

RESULT

Effect of Si on plant growth

As shown in table 1, the dry and fresh weight of shoot and root and fruit of fenugreek was significantly reduced by NaCl stress. It is clear that the majority of variables showed higher values in control treatment. In general, in fenugreek all growth parameter, showed lower values in the NaCl treatment however these growth parameters slightly increased significantly by Si, supplement (table1).

Effect of Si on chlorophyll a

Chlorophyll a contents were lower in plants that grown at high salinity compared to control values, Si completely restored chlorophyll a levels in the salinity treatment, 0, 60, 120 and 180 NaCl treatment presented 5.460, 4.996, 5.145,4.218 mg g^{-1} FM, respectively (table2). The control + 1.5 mM Si was significantly higher than others treatments.

Effect of Si on chlorophyll b

The result obtained on chlorophyll b level prove the silicon action in the Fenugreek under study, in which were showed, 3.951, 3.510, 4.11, 2.898 mg g^{-1} FM in 0,60,120 and 180 mM NaCl treatment, respectively (table2). The experiment revealed that the exogenous silicon increase this pigment statistically higher than control.

Modification in the ratio chlorophylls a/b promoted by Si

The ratio chlorophylls a/b prove the influence of the silicon application in these pigments in which the treatment with 1.5 mM Si + control had higher value and statistically different of all the treatments (table 2). In addition, the ratio presented in the 0, 60, 120, 180 mM NaCl treatments the values of 1.511, 1.501, 1.450, 1.095 mg g^{-1} FM, respectively and the ratio presented in these treatments with 1.5mM Si values of 1.869, 1.479, 1.578, 1.314 mg g^{-1} FM respectively.

Effect of Si on leaf relative water content

Leaf relative water content (RWC) dropped significantly with higher salinity of the irrigation water (63.5% at 60 mM NaCl, 59.92% at 120 mM NaCl and 58.96 at 180 mM NaCl) in plant without Si (table 2). Si completely restored RWC levels in the salinity treatments. Si improves RWC in plants under non saline treatment. The values obtained revealed that silicon promoted the increase of water retention in the leaf tissue under salinity stress.

Effect of Si on Electrolyte leakage

The salinity treatment impaired membrane permeability by increasing electrolyte leakage. Electrolyte leakage (EL) showed slightly higher values with respect to NaCl treatment in Fenugreek. Addition of Si partially maintained membrane permeability (Table 2).

Table 1. Effect of silicon on plant growth (g/plant) in Fenugreek under salinity stress. Results of statistical analysis are also given.
Ns – not significant, * -P= 0.05; ** -P= 0.01

NaCl [mM]	Si [mM]	Root fresh weight [g $plant^{-1}$]	Root dry weight [g $plant^{-1}$]	Shoot fresh weight [g $plant^{-1}$]	Shoot dry weight [g $plant^{-1}$]	Fruit fresh weight [g $plant^{-1}$]	Fruit fresh weight [g $plant^{-1}$]
0	0	10/170 b	1/073 ab	13/170 b	2/110 ab	3/339 ab	0/695 abc
0	1/5	19/030 a	1/541 a	24/290a	3/005 a	4/524 a	1/051 a
60	0	9/230 bc	0/949 b	12/600 b	1/729 b	2/535 bc	0/919 ab
60	1/5	11/490 b	1/063 ab	14/130 b	1/503 b	3/347 ab	0/685 abc
120	0	8/332 bc	0/644 b	10/920 bc	1/172 b	1/790 c	0/509 c
120	1/5	10/120 b	0/970 b	13/130 b	1/408 b	1/656 c	0/632 bc
180	0	4/040 d	0/764 b	55/308 d	1/080 b	1/565 c	0/460 c
180	1/5	4/784 cd	0/651 b	6/260 cd	1/330 b	1/441 c	0/507 c
NOV.	Si	232/902**	0/790 ns	312/591**	1/651 ns	3/568 ns	0/105 ns
	NaCl	350/842**	1/398**	566/130**	7/704*	26/086**	0/684**
	Si × NaCl	68/094*	0/322 ns	115/645*	1/053 ns	2/107 ns	0/294 ns

Table 2. Effect of silicon on chlorophyll contents (mg/g/FM), Electrolyte leakage (%) and Leaf relative water content (RWC) in Fenugreek under salinity stress. Results of statistical analysis are also given. Ns – Not significant, * -P= 0.05; ** -P= 0.01

NaCl [mM]	Si [mM]	Chl. a [mg $g^{-1}FM^{-1}$]	Chl. b [mg $g^{-1}FM^{-1}$]	Chl. a/b [mg $g^{-1}FM^{-1}$]	EL [%]	RWC [%]
0	0	5/460 bcd	3/951 bcde	1/511 abc	32/512 bc	63/670 ab
0	1/5	7/536 a	4/917 ab	1/869 a	28/605 c	66/510 a
60	0	4/996 bcd	3/510 cde	1/501 abc	38/677 ab	63/500 ab
60	1/5	6/049 b	5/217 a	1/479 abc	34/504 bc	62/681 ab
120	0	5/145 bcd	4/118 abcde	1/450 abc	37/784 ab	59/920 bc
120	1/5	5/783 bc	4/606 abc	1/578 ab	36/655 b	58/220 bc
180	0	4/218 d	2/898 e	1/095 c	44/758 a	55/020 c
180	1/5	4/572 cd	3/244 de	1/314 bc	37/39 ab	58/960 bc
ANOVA	Si	21/229**	15/383**	0/584 ns	343/363ns	16/412ns
	NaCl	14/770**	8/682**	0/806*	377/901*	272/742**
	Si × NaCl	2/841ns	1/882ns	0/128ns	32/543ns	39/721ns

DISCUSSION

It has been found by various workers that silicon has positive effects on the growth and yield as well physiology and metabolism of different crops. In this experiment, it has been shown that salt stress in Fenugreek causes very significant reduction in all growth variables, including fresh and dry weight, these findings are in agreement with the work of Navarro et al (2000) and kaya et al (2001) for tomato. On the other hand, supplementary silicate resulted in slightly increased plant growth variables (Table1), similar results were found in other crops such as rice (yeo et al., 1999), Gerbera (savvas et al., 2002). Supplementary Si resulted in significant increase in chlorophyll contents of plants grown at high NaCl. Similar results were observed in cucumber and tomato (Miyake, 1992) and barley (liang, 1999). The ratio chlorophyll a/b proves the influence the silicon application in these pigments, (Table 2) the treatment of control with 1/5Mm Si had higher value and statistically different of all the treatments (Table 2). The chlorophyll a level was maintained in the treatment that was applied 1/5Mm Si. It in agreement with liang et al (1996). The silicon decreases the permeability of plasma membrane of leaf cells, as well as this element improves the ultrastructure of the chloroplasts (liang, 1998). The chlorophyll a is considered as the main photosynthetic pigment in higher plants, in which this pigment is responsible by the light absorption that promotes the start of the photosynthesis process (Taiz and zeiger, 2002). The maintenance of the chlorophyll b of the plants under salinity + silicon was probably promoted by the increase of antioxidant enzyme activities as super oxide dismutase and catalase (Gong et al., 2005). These enzymes had the function to avoid the oxidative stress provoked during situation of a biotic or biotic stress (Liang et al., 2003). The maximization in ratio chlorophylls a/b of the plants submitted to silicon is directly linked with the benefits showed in chlorophylls a and b, in which this ratio indicates the light absorption, was increased. Membrane permeability was determined by measuring electrolyte leakage. High NaCl treatment (180Mm) induced significant increases in electrolyte leakage compared with the control plants (table 2) similar results were obtained by lutts et al (1996) and kaya et al (2002). The results obtained from Electrolyte leakage measurements showed that silicate may act to alleviate salt stress in fenugreek by slightly decreasing the permeability of the membrane to help these structures maintain their form (Table 2), similar results were found by liang et al and liang (1998). The leaf relative water content is normality used to evaluate the water volume/ amount presents in plant tissue. The leaf relative water content of plants submitted to external silicon were kept in higher levels than the plants under stress as well as this study reveals the benefit effects of silicon. The results obtained in this study promoted by silicon are corroborated by the experiment conducted by Ran ganathan et al (2006).

CONCLUSION

The result obtained on water relations and pigments indicated that the silicon increased the tolerance to salinity stress, in which the plant growth, leaf relative water content, chlorophylls a and b, were maintenance in higher levels if compared with stress plant. This way, this study corroborates the benefice effects of the silicon exogenous application in fenugreek plants under salinity stress.

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