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BRIEF COMMUNICATION

Effects of silicon on photosynthesis, water relations and nutrient uptake of *Phaseolus vulgaris* under NaCl stress

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Abstract

A greenhouse experiment was conducted to investigate the effects of silicon application on *Phaseolus vulgaris* L. under two levels of salt stress (30 and 60 mM NaCl in the irrigation water). Salinity significantly reduced growth, stomatal conductance and net photosynthetic rate, and increased Na^+ and Cl^- content mainly in roots. Silicon application enhanced growth of salt stressed plants, significantly reduced Na^+ content especially in leaves and counterbalanced the effects of NaCl on gas exchange; the effect was more evident at 30 mM NaCl. Cl^- content in shoots and roots was not significantly modified by silicon application; the drop in K^+ content caused by salinity was partially counterbalanced by silicon, especially in roots.

Additional key words: apoplast, net photosynthetic rate, salt stress, stomatal conductance.

Silicon is the second most abundant element on earth, yet its role in plant biology and physiology has not been understood clearly. It is not counted among the essential elements for higher plants, apart from some species belonging to *Poaceae* and *Cyperaceae* (Epstein 1994). Nevertheless it is commonly accepted that silicon can positively affect growth and health status of plants under biotic (Adatia and Besford 1986, Menzies *et al.* 1991, Ma 2004) and abiotic (Barceló *et al.* 1993, Iwasaki *et al.* 2002, Ranganathan *et al.* 2006) stresses.

Satisfactory results of silicon application against NaCl stress have been shown in rice (Matoh *et al.* 1986), wheat (Ahmad *et al.* 1992), *Prosopis juliflora* (Bradbury and Ahmad 1990) and barley in hydroponics (Liang *et al.* 1996, Liang 1998, 1999, Liang and Ding 2002). Possible explanations for this induction of tolerance were proposed, *e.g.* *1*) accumulation of silicon in leaves limiting the transpiration (Matoh *et al.* 1986), *2*) formation of complexes with Na in roots (Ahmad *et al.* 1992), *3*) protection of plasmatic membranes and chloroplasts ultrastructure (Liang *et al.* 1996, Liang 1998), *4*) stimulation of the activity of H^+-ATPase (Liang *et al.* 1996, 2003), and *5*) protection of plant tissues from free radicals through an increase in the activity of antioxidative enzymes (Liang 1999, Liang *et al.* 2003). An alternative explanation has been given by Yeo *et al.* (1999), hypothesizing that silicon limits the portion of water passing through the root by apoplastic way, so reducing the entrance of Na^+ without significantly affecting the overall transpiration flux and plant growth.

Aim of this work was to examine the effects of silicon application on *Phaseolus vulgaris* L. under saline stress with particular attention to gas exchange and ion uptake.

The experiment was conducted in PVC greenhouse (temperature 18 °C minimum; 29 °C maximum, relative humidity between 50 and 70 %; maximum midday photosynthetic photon flux density, PPFD, measured with *Quantometer Radiation Sensor*, *Li-COR*, UK, ranging from 500 μmol m^{-2} s^{-1} on days with heavy cloud cover to 1200 μmol m^{-2} s^{-1} on clear days; natural light was supplemented from 09:00 to 19:00 h with additional irradiation provided by 400 W metal halide bulbs, PPFD 200 μmol m^{-2} s^{-1}). Seedlings of *Phaseolus vulgaris* L. cv. Navy were transplanted at the 10^{th} day from sowing into plastic pots with the capacity of 1750 cm^3 filled with a mixture of peat:vermiculite 1.5:1 (v/v). The model species was chosen for the rapidity of its biological cycle and for its medium-high tolerance to salinity

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Abbreviations: g_s - stomatal conductance; P_N - net photosynthetic rate; RWC - relative water content.
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(Maas and Hoffmann 1977). Six different treatments were compared, deriving from the factorial combination of three levels of salinity of the irrigation water and the presence or absence of silicon. Each combination of conditions was applied to a set of 10 plants, and replicated 3 times. Pots were randomized daily. Plants were drip-irrigated with 10 % Hoagland solution in order to avoid excessive accumulation of nutrients in the solid substrate. Salinity treatments (0, 30 and 60 mM NaCl) started from the 6th day after potting and went on until the end of the trial. Silicon was added to the nutrient solution as K_2SiO_3 (0 and 1.5 mM). KNO_3 was reduced, in the preparation of the Hoagland solution, proportionally to the K supply provided by K_2SiO_3, and the loss of NO_3^- was subsequently reintegrated by addition of nitric acid. The pH of the solution was daily adjusted to 6.5 - 7.0 with 0.01 M KOH and 0.01 M H_2SO_4. The increase in electrical conductivity caused by the addition of K_2SiO_3 was negligible.

Dry mass of shoots was measured after their drying in oven at 75 °C till constant mass. Leaf area was measured with area meter *MK2*, *Delta T-Devices*, Burwell, UK. Relative water content (RWC) of the four most expanded leaves of each plant was measured gravimetrically. Stomatal conductance (g_s) and net photosynthetic rate (P_N) were measured on the two most expanded leaves of each plant at the beginning, in the middle and at the end of the treatment period (days 0, 7 and 14, corresponding to days 6, 13 and 20 from potting) with a *Li-6400* portable photosynthetic system (*Li-COR*), and the measurement repeated twice. The CO_2 concentration in the chamber (area 6 cm^2) was 360 $\mu g\, g^{-1}$, PPFD was 1100 - 1200 $\mu mol\, m^{-2}\, s^{-1}$, provided by a *6400-02 LED*. At the end of the trial roots and shoots of plants were dried in oven, then ground and digested with nitric acid. Na^+ and K^+ contents were determined with flame spectrometry (*Perkin Elmer mod. 290B*, Norwalk, USA), Cl^- content with the Zall method (Zall *et al.* 1956). Data were subjected to analysis of variance using the *SAS* statistical software program: two-way *ANOVA* was used to determine significant differences among different treatments grouped on the basis of salinity level and presence or absence of silicon. Least significant difference (LSD) was calculated at 0.05 probability level for each parameter.

Table 1. Effects of silicon and NaCl on stomatal conductance [mmol(H_2O) $m^{-2}\, s^{-1}$] and net photosynthetic rate [μmol(CO_2) $m^{-2}\, s^{-1}$] measured 16, 23 and 30 d after treatment. Results of statistical analysis are also given. NS - not significant, * - P = 0.05; ** - P = 0.01; *** - P = 0.001.

NaCl [mM]	Si [mM]	Day 16 g_s	Day 16 P_N	Day 23 g_s	Day 23 P_N	Day 30 g_s	Day 30 P_N
0	0	637	20.06	610	21.02	629	20.09
0	1.5	660	19.09	673	21.03	640	21.05
30	0	695	20.03	429	15.00	387	14.06
30	1.5	647	20.06	561	18.09	535	17.05
60	0	663	20.05	277	14.01	231	10.04
60	1.5	667	20.03	403	15.03	369	14.02
$LSD_{0.05}$		50	0.13	79	2.64	115	3.10
ANOVA	Si	0.5 NS	0.3 NS	9.6***	7.1**	19.7***	7.5**
	NaCl	0.1 NS	0.5 NS	20.3***	9.2**	42.0***	21.4***
	Si×NaCl	0.1 NS	0.3 NS	0.2 NS	1.7 NS	3.5*	2.0 NS

Table 2. Effects of silicon and NaCl on shoot dry mass, leaf area, RWC and Na^+, Cl^- and K^+ content in leaves and roots measured 30 d after treatments. Results of statistical analysis are also given. NS - not significant, * - P = 0.05; ** - P = 0.01; *** - P = 0.001.

NaCl [mM]	Si [mM]	Shoot d.m. [g $plant^{-1}$]	Leaf area [%]	RWC [cm^2]	Na^+ [$\mu mol\, g^{-1}$(d.m.)] leaves	Na^+ roots	Cl^- [$mmol\, g^{-1}$(d.m.)] leaves	Cl^- roots	K^+ [$mmol\, g^{-1}$(d.m.)] leaves	K^+ roots
0	0	8.15	83	2214	130	78	0.22	0.07	1.20	1.57
0	1.5	8.05	86	2076	52	45	0.19	0.11	1.14	1.53
30	0	7.05	70	1799	360	480	1.06	0.43	1.01	1.16
30	1.5	8.10	87	1906	201	303	1.12	0.40	1.07	1.40
60	0	5.50	45	1220	740	957	2.01	0.81	0.77	0.81
60	1.5	6.15	61	1736	413	790	1.94	0.75	0.99	1.29
$LSD_{0.05}$		0.57	14	210	75	120	0.15	0.13	0.21	0.30
ANOVA	Si	31.2***	9.4**	24.5***	21.7***	9.9**	2.5*	3.4*	6.3*	4.8*
	NaCl	14.5***	15.2***	51.3***	38.0***	18.6***	2.9*	7.1**	9.3**	11.3**
	Si×NaCl	0.7 NS	1.2 NS	0.5 NS	2.3 NS	3.8*	0.5 NS	1.4 NS	3.2*	1.8 NS

Stomatal conductance decreased after 23 or 30 d of NaCl treatment. Silicon treated plants showed similar patterns to non-treated ones, but g_s was mostly higher, particularly at 30 mM NaCl. Photosynthetic rate followed the same patterns as stomatal conductance. The effect of silicon supply in limiting the decrease of photosynthetic rate was particularly relevant at 30 mM NaCl (Table 1).

Si application generally counterbalanced growth reduction caused by salinity (Table 2). Compensation for shoot dry mass was total at 30 mM NaCl, while at 60 mM growth of Si treated and non-treated plants was reduced by 14.3 and 32.5 %, respectively. Si treated plants showed also slighter reductions of leaf area (-8.1 instead of -20.5 % at 30 mM NaCl; -16.3 instead of -44.8 % at 60 mM NaCl). However, at 0 mM NaCl Si treated plants showed lower leaf area.

Leaf RWC dropped significantly with higher salinity of the irrigation water (-15.7 % at 30 mM NaCl; -45.8 % at 60 mM NaCl) in plants without Si (Table 2). The application of Si totally (at 30 mM NaCl) or partially (at 60 mM NaCl) alleviated the drop of RWC (Table 2).

Tissue content of Na^+ increased considerably in response to salt treatments (Table 2). The application of Si reduced significantly Na^+ content both in shoots and roots at all salinity levels including 0 mM NaCl, with a stronger effect in shoots. Under salinity, all plants showed on the average higher levels of Na^+ in roots than in shoots. Cl^- content was increased by salt treatments in both parts, with a preferential allocation in roots, and in no case it was affected by Si application. Exposure to salt stress caused the drop of K^+ content, especially in roots. Si supply partially counterbalanced this effect, limiting the K^+ reduction particularly at roots level and showing the most remarkable effect at 60 mM NaCl.

The experiment has shown how the addition of Si to the irrigation water can ameliorate negative effects NaCl on growth and on important ecophysiological parameters connected with it, such as g_s, P_N and RWC, as well as reduce Na^+ and Cl^- content in plant tissues. These results are in agreement with the theory according to which silicon could limit salt stress by partially blocking the apoplastic transport, which is responsible for the entry of the main part of Na^+ through plant roots (Yeo *et al.* 1987, Yadav *et al.* 1996, Garcia *et al.* 1997). Moreover, practically no influence was observed when silicon was supplied to non-stressed plants, similarly as shown Matoh *et al.* (1986) in rice. The close connection between reduction of P_N and g_s in salt stressed was already shown by Yeo *et al.* (1985), Brugnoli and Lauteri (1991), Stępień and Kłobus (2006). Si application under salt stress increases the both parameters.

Salt stress acts on leaves both in terms of dimension and of total biomass. Si application partially counterbalances this effect, acting more positively on biomass production than on leaf size (Bursleim *et al.* 1996).

In conclusion, the use of Si resulted in reduction of salt stress, especially by means of limiting Na^+ accumulation in leaves. Their higher RWC caused improved g_s and P_N under NaCl stress and in consequence an increased dry mass production.

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