Progress in Biological Sciences

Vol. 4, Number 2, Summer / Autumn 2014/189-202

Silicon alleviates salt stress in pistachio plants

Received: 1 February, 2014; Accepted: 13 May, 2014

Ghader Habibi[1]*, Fahimeh Norouzi[2], Roghieh Hajiboland[2]

1. Department of Biology, Payame Noor University, I. R. of Iran

2. Department of Plant Science, University of Tabriz, 51666-14779 Tabriz, Iran

ABSTRACT

In this work, the effects of silicon (Si) supplementation were studied in pistachio (*Pistacia vera* L. cv Ahmadaghaii) plants exposed to high salinity stress. Plants were grown in pots under control and salt (EC=15 dS m^{-1}) conditions without or with Si treatment (0.35 g Na_2SiO_3 Kg^{-1} soil) under field conditions. Salt stress reduced the plants' growth significantly in both –Si and +Si plants; however, Si-supplied plants had a higher root and shoot dry weight as compared to those without Si supply under salinity conditions. Salt stress caused a significant reduction of leaf photochemical activities; however, Si application ameliorated these effects. The reduction of the net CO_2 assimilation rate under salinity stress was alleviated by Si application, accompanied by an increase in water-use-efficiency. The concentration of Na in the leaves and roots was significantly reduced by Si, while root K and leaf Ca concentrations were higher in Si-treated plants under salt stress compared with –Si ones. The activity of antioxidative enzymes increased under salt stress and Si application caused a further increase, being significant for superoxide dismutase (SOD). Salt stress induced membrane damage, as was indicated by a higher malondialdehyde (MDA) concentration. In Si-supplemented plants, however, the MDA amount did not increase under salt stress. The results indicated that the Si-mediated alleviation of salt stress in pistachio plants is related to higher photosynthesis and water-use efficiency, a reduction of Na uptake and transport, and the stimulation of the plant's antioxidative defence capacity.

Keywords: antioxidant defence system, net CO_2 assimilation rate, *Pistacia vera*, salinity, sodium silicate.

* **Corresponding author:** gader.habibi@gmail.com

Introduction

Salinity is the main stress factor limiting plant growth and productivity (1, 2). About 7% of the earth's total land area is affected by salt. In addition, the salinization of irrigated agricultural land is becoming a major problem for food production in arid and semiarid regions of the world (3). High concentrations of salts cause both hyperionic and hyperosmotic stresses, and can lead to a decrease in plant productivity or else death (4).

Similar to other environmental stresses, salt stress leads to the generation of reactive oxygen species (ROSs), such as superoxide radical (O_2^-), hydroxyl radical (OH), singlet oxygen ($^1O_2$) and hydrogen peroxide (H_2O_2) (5). On the other hand, because of a reduction in the availability of atmospheric CO_2 following stomatal closure, the consumption of NADPH by the Calvin cycle diminishes, which initiates chain reactions and the production of ROSs (6). The accumulation of ROSs damages critical organelles via lipid peroxidation and is capable of inducing damage to almost all cellular macromolecules, including DNA, proteins and carbohydrates (7). The activity of antioxidative enzymes as the most important components in scavenging and the prevention of ROSs damage usually increases under salt stress conditions (8, 9). Accordingly, a correlation exists between the activity of antioxidant enzymes and the salt tolerance of plants (10).

A high salinity enhances the osmotic potential of the soil matrix, which limits plants' water uptake. In addition, Na^+ at toxic concentrations interferes with K^+ uptake, resulting in alterations in ionic homeostasis. Thus, the accumulation of Na and the reduction of K and Ca uptake and translocation in salt-stressed plants result in severe metabolic disturbances in plants (11).

Silicon (Si) is accumulated in plants at a rate comparable to those of macronutrients, such as Ca, Mg and P (12). Silicon application has been reported to enhance the tolerance of crop species to salinity stress (13). The ameliorative role of Si under salinity has been reported in different plant species, such as barley (13), wheat (14) and tomato (15).

Possible mechanisms of the Si-mediated alleviation of salt stress in higher plants include:

i) the stimulation of enzymatic and non-enzymatic antioxidative defence systems (16) and the reduction of oxidative membrane damage (17),

ii) the improvement of water uptake via the increased volume and weight of roots (18), the prevention of water loss via the reduction of both cuticular (19) and stomatal transpiration, and

iii) the reduction of Na^+ uptake (20) and an increasing K:Na ratio (21) and/or the alteration of Na^+ distribution and other ions within plants.

It has been demonstrated that Si application in wheat plants increases the binding of Na to the cell wall (CW), and thus results in the reduction of potentially toxic leaf sap Na^+ concentrations (11). However, information is lacking on the effect of Si treatment on the Na distribution among cell compartments in other species.

Pistachio (*Pistacia vera* L.) plants are one of the most important tree crops in the Mediterranean climate (22) and in Iran (23). Pistachio is a salt-tolerant species (22). The area of the pistachio orchards of Iran is more than 360,000 ha, with about 13 million pistachio trees (23) comprising 60 different varieties. In our previous work (24), it was demonstrated that the supplementation of water-deficient pistachio plants with Si

alleviates the adverse effects of drought stress. An enhancement of leaf photochemical efficiency and photosynthetic gas exchange, as well as the activation of the antioxidant defence capacity of plants, have been mechanisms for Si-mediated growth improvement in drought-stressed pistachio plants.

Studies on the effects of Si supplementation in salt-stressed plants have mainly focused on relatively salt-sensitive and/or moderately-tolerant species, such as wheat (11, 14), maize (25) and canola (16). However, the responses of highly-tolerant species such as pistachio to Si application have not been characterized thus far.

This work is aimed at studying the effect of Si added to the soil on dry matter production, antioxidative defence and ion relations in pistachio plants. In addition, in order to characterize the effect of Si on Na accumulation and subcellular distribution, leaf tissues were subjected to a fractionation procedure, and the Na distribution between CW-bound and free fractions as affected by Si treatments were analysed in pistachio leaves.

Materials and Methods

Plant growth and treatments

Seeds of pistachio (*Pistacia vera* L. cv Ahmadaghaii) were sown in the top of cylindrical plastic pots - four seeds were planted in each pot. The pots were 14 cm in diameter and 105 cm in depth, filled with 15 kg sandy loam soil (pH 7.6). For the basal fertilization, 200 mg nitrogen kg^{-1} soil as NH_4NO_3 and 50 and 62.5 mg phosphorus and potassium kg^{-1} soil as KH_2PO_4 were applied. Before filling the pots, the soils of the Si treatments were fertilized with 0.35 g sodium metasilicate (Na_2SiO_3) kg^{-1} soil (3.44 mmol dm^{-3} soil ≈ 2.73 mmol kg^{-1} soil).

After emergence, the seedlings were thinned to one plant per pot and irrigated with distilled water every five days to maintain at 90% field capacity (FC). Seven weeks after sowing, salinity treatments were started. The pots were assigned randomly to control or salt treatments and NaCl was added to the latter group in order to achieve the electric conductivity (EC) of 15 dS m^{-1} according to the method described by Hajiboland et al. (26). The EC of the control pots was 1.32 dS m^{-1}.

Plants were grown under field conditions located near the city of Miandoab, NW Iran (46º6’ E and 36º46’ N) with a day/night temperature of 20-35/17-20 ºC, a relative humidity of 35-45% and a daily photon flux density (PFD) of about 1,200-1,700 µmol/m^2 s throughout the experimental period.

Plant harvest and analysis of growth parameters

Plants with four-to-five leaf pairs (14 weeks after sowing, seven weeks after salt treatment) were harvested. The leaves and roots were separated and washed with distilled water, blotted dry on filter paper and, after the determination of fresh weight (FW), dried for 48 h at 70 °C for the determination of dry weight (DW).

Measurements of chlorophyll fluorescence parameters and photosynthetic gas exchange

Chlorophyll (Chl) fluorescence parameters were recorded using a portable fluorometer (OSF1, ADC Bioscientific Ltd., UK) for both dark-adapted and light-adapted leaves. The leaves were acclimated to dark for 30 min using leaf clips before measurements were taken. The initial (F_0), maximum (F_m) and variable (F_v=F_m-F_0) fluorescence as well as the maximum quantum yield of PSII (F_v/F_m)

were recorded. The light-adapted leaves were used for the measurement of the steady-state (F_s) and maximum (F'_m) fluorescence. Calculations were made for F'_0 ($F'_0=F_0/[(F_v/F_m)+(F_0/F'_m)]$), photochemical quenching, qP $[(F'_m-F_s)/(F'_m-F'_0)]$ and non-photochemical quenching, qN $(1-[(F'_m-F'_0)/(F_m-F_0)])$ (27).

The net CO_2 fixation (A, μmol m^{-2} s^{-1}), transpiration rate (E, mmol m^{-2} s^{-1}) and stomatal conductance to water vapour (g_s, mol $m^{-2}s^{-1}$) were measured with a calibrated portable gas exchange system (LCA-4, ADC Bioscientific Ltd., UK) after 5 h into the light period under a photon flux density of about 1,500 μmol m^{-2} s^{-1}.

Assay of enzyme activity and related metabolites

The activity of superoxide dismutase (SOD, EC 1.15.1.1), peroxidase (POD, EC 1.11.1.7), catalase (CAT, EC 1.11.1.6) and ascorbate peroxidase (APX, EC 1.11.1.11) were determined according to the methods described elsewhere (28). The activity of the enzymes was determined in leaves harvested in the middle of the day. Lipid peroxidation was estimated from the amount of malondialdehyde (MDA) formed in a reaction mixture containing thiobarbituric acid. The soluble protein was estimated by the Bradford method (29).

The hydrogen peroxide (H_2O_2) contents in the leaves were assayed according to the method of Velikova et al. (30). The leaves were homogenized in an ice bath with 0.1% (w/v) TCA. The extract was centrifuged at 12,000 × g for 15 min, after which 0.5 ml of the supernatant was added to 0.5 ml of 10 mM of a potassium phosphate buffer (pH 7.0) and 1 ml of 1 M KI; the reaction was improved for 1 h in the dark and measured spectrophotometrically at 390 nm. The content of the H_2O_2 was given on a standard curve.

Determination of ions and Si content

The leaf and root samples were transferred to porcelain crucibles and dry-ashed at 550 °C for 8 h, resolved in 0.5 M HCl and made up to volume by double-distilled water. Concentrations of Na, Ca and K were determined by a flame-photometer (Jenway, PFP7).

The leaves and roots were prepared for the determination of Si (31) using inductively-coupled plasma-atomic emission spectrometry (ICP-AES, INTEGRA XL2, GBC, Australia).

Isolation of CW and analysis of CW-bound Na

The cell walls were isolated following the method of Saqib et al. (11). The leaf samples were cut into small pieces and homogenized in a hypertonic sucrose solution (0.4 M). The homogenate was centrifuged at 700 g at 4°C and thereafter the pellet/cell walls were washed in increasing concentrations of sucrose (with 0.4 M sucrose, 0.6 M sucrose and 1 M sucrose), 0.1% (v/v) Triton X-100, and finally distilled water. The free Na concentration was determined in sucrose, Triton X-100 and distilled water washing solutions. The isolated cell walls were further washed by centrifugation, successively with $CaCl_2$ (50 mM) and 0.5 mM HCl, and thereafter oven-dried at 70°C for 48 h. This cell wall material was digested in 5 M HNO_3. The Na concentration was determined in $CaCl_2$ and HCl washing solutions, as well as in the digested cell wall in HNO_3 by inductively-coupled plasma-atomic emission spectrometry. Sodium in the total fraction was considered as the CW-bound Na analogously to Mn according to Rogalla and Römheld (32).

The experiment was performed in a

complete randomized block design with four pots as four independent replications. Statistical analyses were carried out using sigma stat (3.5) with Fisher's LSD test (P< 0.05).

Results

Salt stress significantly reduced the root and shoot fresh and dry weights in pistachio plants in the absence or presence of Si (Fig. 1). Silicon-supplied plants, however, showed higher root and shoot dry weights compared with plants without Si treatment under salinity conditions (Fig. 1). In the absence of salt, the fresh and dry weight of the roots was higher in Si-treated plants compared with control plants. The results of two-way ANOVA showed that the effect of either salt or Si treatment alone was significant for the dry weight of both roots and shoots, while the salinity × Si interaction effect was not statistically significant (Table 1).

Table 1. Results of a two-way ANOVA test (mean of squares) for the effect of salinity and Si and their interactions with various physiological parameters in pistachio plants

Parameters	Salinity	Si	Salinity× Si
Shoot FW	2.83***	0.041 ^{ns}	0.13 ^{ns}
Shoot DW	0.124***	0.016**	0.006 ^{ns}
Root FW	0.931***	0.148*	0.004 ^{ns}
Root DW	0.065***	0.006**	0.000 ^{ns}
F_o	1560 ^{ns}	4.00 ^{ns}	1.00 ^{ns}
Fm	115770 **	9850 ^{ns}	17358 ^{ns}
qN	0.049 **	0.001 ^{ns}	0.000 ^{ns}
F_v/F_m	0.038***	0.002 ^{ns}	0.004 ^{ns}
A	18.1***	3.04**	1.62 *
g_s	0.15***	0.001^{ns}	0.001 ^{ns}
E	0.660***	0.007 ^{ns}	0.008 ^{ns}
WUE	0.48 ^{ns}	5.62***	3.24 **
Ci/Ca	0.001 **	0.001**	0.000 **
Leaf free Na	1537***	144**	99.8*
Leaf bound Na	93.1***	0.260 ^{ns}	15.3 ^{ns}
Leaf total Na	3336***	132**	36.9 ^{ns}
Root Na	18852***	1560 ^{ns}	4778*
Leaf K	283.4 ^{ns}	444.3 ^{ns}	235.8 ^{ns}
Root K	296.9 ^{ns}	9599***	305.6 ^{ns}
Leaf Ca	9.71 ^{ns}	31.0**	4.24 ^{ns}
Root Ca	0.022**	0.001 ^{ns}	0.015*
Leaf Si	2.44 ^{ns}	251***	2.62 ^{ns}
Root Si	8.94 ^{ns}	602***	6.83 ^{ns}
SOD	758***	59.2*	60.8*
POD	0.216***	0.002 ^{ns}	0.001 ^{ns}
CAT	2796***	65.4 ^{ns}	115 ^{ns}
APX	0.325***	0.005^{ns}	0.001 ^{ns}
MDA	347***	176**	240 **
H_2O_2	1.31***	0.00 ^{ns}	0.002 ^{ns}

ns: non-significant, according to the Fisher LSD test, *** *P*< 0.001, ** *P*< 0.01, * *P*< 0.05.

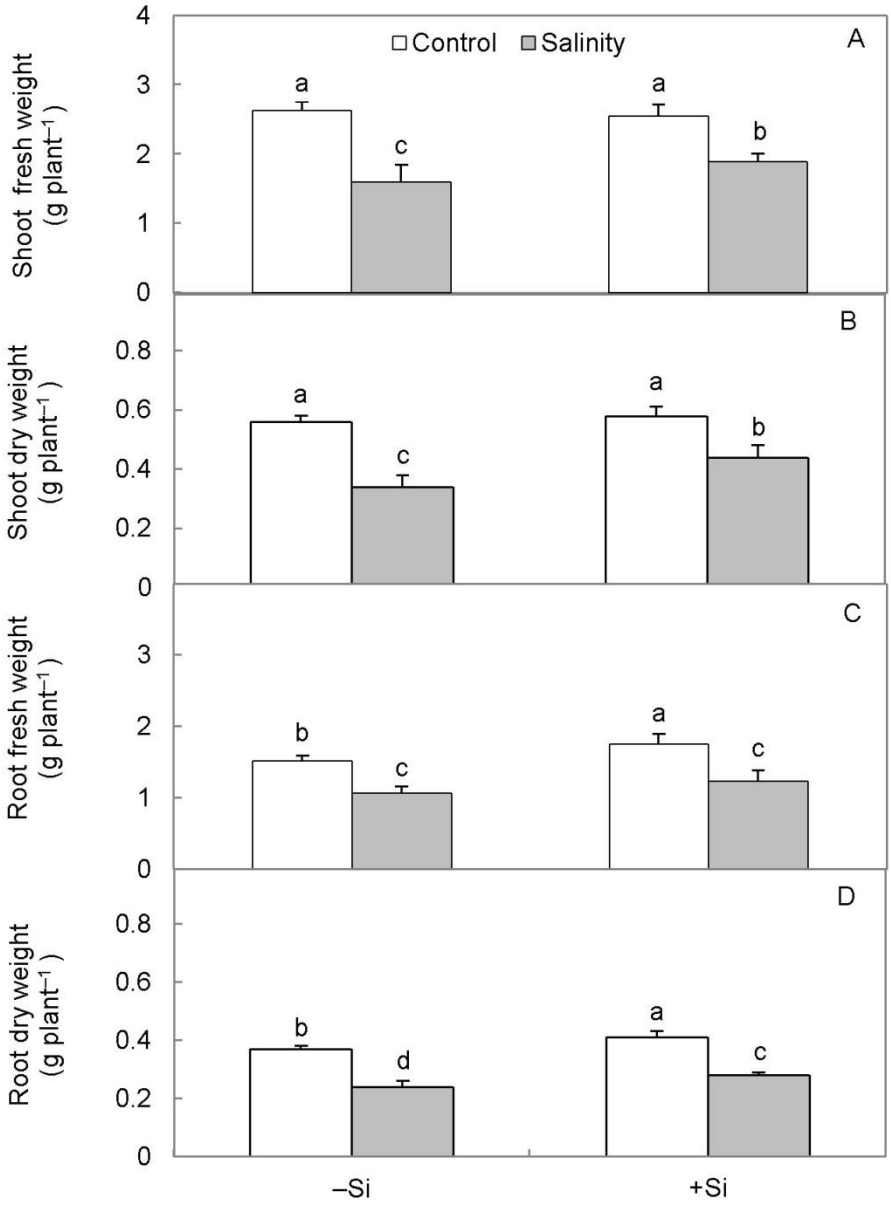

Figure 1. Fresh weight (FW) and dry weight (DW) of shoots (A, B) and roots (C, D) in pistachio plants grown for seven weeks under saline conditions without or with the application of Si. The data are the mean ± SD of four replicates. Bars indicated with the same letter are not significantly different (P< 0.05).

No significant differences were found between –Si and +Si plants in the minimum Chl fluorescence (F_0) and photochemical quenching (qP) of leaves (Table 2). However, salt-treated plants tended to have a lower maximum Chl fluorescence yield (F_m) than the control plants without salt. Salt stress resulted in higher non-photochemical quenching (qN) in the leaves, while the Si-treated plants did not differ from the –Si plants in this regard. The maximum efficiency of PSII (F_v/F_m) exhibited a decrease in those plants subjected to salinity in the absence or presence of Si. The extent of the reduction, however, was lower in the +Si plants and, in consequence, this parameter was significantly higher in salt-treated plants under Si application.

Table 2. Changes in the minimum fluorescence of Chl (F_0), maximum fluorescence yield (F_m), photochemical quenching (qP), non-photochemical quenching (qN), maximum quantum yield of PSII (F_v/F_m), net photosynthetic rate (A, µmol m^{-2} s^{-1}), transpiration rate (E, mmol m^{-2} s^{-1}), stomatal conductance (g_s, mol m^{-2} s^{-1}), water-use efficiency (WUE (A/E), µmol $mmol^{-1}$) and the ratio of the CO_2 concentration in the intercellular space to ambient air (C_i/C_a), in pistachio plants grown for seven weeks under saline conditions without or with the application of Si. The data are the mean ± SD of four replicates. The data of each column indicated by the same letter are not significantly different ($P < 0.05$).

Treatments		Photochemistry				
		F_0	F_m	qP	qN	F_v/F_m
−Si	Control	156 ± 10.2 [a]	774 ± 35.0 [a]	0.89 ± 0.05 [a]	0.11 ± 0.07 [b]	0.79 ± 0.01 [a]
	Salinity	175 ± 25.0 [a]	538 ± 146 [b]	0.94 ± 0.08 [a]	0.23 ± 0.05 [ab]	0.67 ± 0.04 [c]
+Si	Control	155 ± 10.0 [a]	758 ± 42.4 [a]	0.88 ± 0.05 [a]	0.13 ± 0.10 [b]	0.79± 0.01 [a]
	Salinity	177 ± 25.7 [a]	654 ± 119 [ab]	0.90 ± 0.07 [a]	0.24 ± 0.03 [a]	0.72 ± 0.04 [b]
		Gas exchange				
		A	g_s	E	WUE	C_i/C_a
−Si	Control	4.95 ± 0.20 [a]	0.34 ± 0.02 [a]	0.88 ± 0.03 [a]	5.64 ± 0.36 [bc]	0.92 ± 0.01 [a]
	Salinity	2.18 ± 0.73 [c]	0.12 ± 0.04 [b]	0.43 ± 0.13 [b]	5.09 ± 0.57 [c]	0.91 ± 0.01 [ab]
+Si	Control	5.18 ± 0.33 [a]	0.33 ± 0.03 [a]	0.86 ± 0.04 [a]	5.93 ± 0.47 [b]	0.90 ± 0.01 [b]
	Salinity	3.69 ± 0.27 [b]	0.15 ± 0.01 [b]	0.52 ± 0.02 [b]	7.18 ± 0.59 [a]	0.88 ± 0.01 [c]

Under salt stress, the net assimilation rate (A), transpiration rate (E) and stomatal conductance (g_s) decreased significantly (Table 2). However, an increase in A was observed in salt-stressed plants upon Si application. The same was observed for the transpiration rate but not for stomatal conductance. Si-applied plants had a higher water-use efficiency (WUE) under salt stress conditions. The ratio of the intercellular CO_2 concentration to that in ambient air (C_i/C_a) was significantly reduced in salt-stressed plants upon Si treatment.

Leaf and root Na concentrations were significantly higher in salt-treated plants regardless of Si treatment. The addition of Si, however, significantly decreased the Na concentrations in the leaves and roots of salinized plants (Table 3).

In plants grown with added salt, leaf K concentrations decreased by 41% (Table 3). The enrichment of the NaCl-containing soil with Si caused a 45% increase in root K content, in comparison with the plants without the addition of Si. However, the shoot K content was not influenced by Si treatment in NaCl-treated plants.

In the roots, the Ca concentration decreased by about 50% in the presence of salt, but was not significantly affected by Si. However, the leaf Ca concentration was higher in salt-stressed plants supplemented with Si compared with other treatment combinations. As expected, the Si concentration of leaves and roots was significantly higher in Si-supplied plants (Table 3). Salinity treatment did not affect Si concentrations. In addition, the constitutive amounts of Si were considerably higher in the roots than in the leaves, irrespective of the treatments. Free and CW-bound Na concentrations were expectedly higher in salt-treated plants in the absence or presence of Si. However, the concentration of free Na was lower in Si-treated plants while the Na concentration in the CW-bound fraction was not influenced by Si (Fig. 2).

Table 3. Concentration of Na, K, Ca and Si (mg g^{-1} DW) in the leaves and roots of pistachio plants grown for seven weeks under saline conditions without or with the application of Si. The data are the mean ± SD of four replicates. The data of each column within each element indicated by the same letter are not significantly different ($P < 0.05$).

Treatments		Leaves	Roots
		Na	
−Si	Control	9.93 ± 1.39 [c]	18.2±7.2 [c]
	Salinity	41.8 ± 2.94 [a]	99.2±11.9 [a]
+Si	Control	7.20 ± 1.89 [c]	29.3±9.3 [c]
	Salinity	33.1 ± 5.46 [b]	53.3±12.2 [b]
		K	
−Si	Control	92.3±22.3 [a]	36.1±6.14 [bc]
	Salinity	38.3±1.10 [b]	26.9±12.0 [c]
+Si	Control	36.4±6.75 [b]	85.4±15.2 [a]
	Salinity	36.9±5.48 [b]	57.0±1.37 [b]
		Ca	
−Si	Control	1.68±0.08 [b]	0.34±0.07 [a]
	Salinity	1.63±0.26 [b]	0.17±0.02 [b]
+Si	Control	1.48±0.23 [b]	0.23±0.07 [ab]
	Salinity	2.58±0.36 [a]	0.18±0.06 [b]
		Si	
−Si	Control	0.79 ± 0.27 [b]	1.13 ± 0.19 [b]
	Salinity	0.50 ± 0.25 [b]	1.32 ± 0.62 [b]
+Si	Control	7.65 ± 1.34 [a]	12.1 ± 3.80 [a]
	Salinity	9.51 ± 3.65 [a]	14.9 ± 3.70 [a]

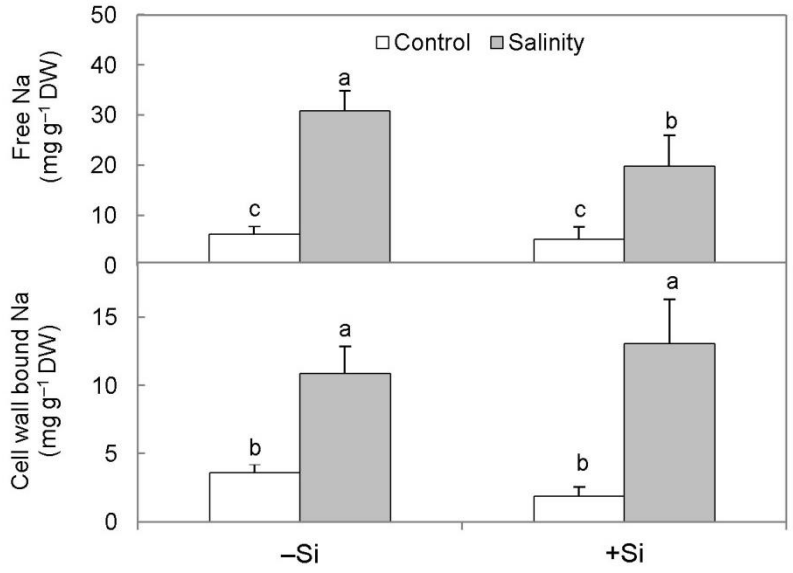

Figure 2. Concentration (mg g^{-1} DW) of free and CW-bound Na in the leaves of pistachio plants grown for seven weeks under saline conditions without or with the application of Si. Each value is the mean ± SD of four replicates. Bars indicated with the same letter are not significantly different ($P < 0.05$).

The antioxidative defence system was influenced by salinity treatment (Table 4). Salt stress significantly increased SOD, POD and CAT activities, while APX activity was not influenced by salt. Si application did not change the activities of CAT, POD and APX, whether in the control or the salt-affected plants. However, a significant increase in the activity of SOD was observed upon Si treatment in salt-stressed plants. Salt stress in the absence of Si caused a significant accumulation of malondialdehyde (MDA) and hydrogen peroxide (H_2O_2). Si treatment had no effect on H_2O_2 concentrations. In contrast, the application of Si decreased the MDA concentration in salt-treated plants (Table 4).

Table 4. Specific activity of superoxide dismutase (SOD, U mg^{-1} protein), catalase (CAT, µmol mg^{-1} protein min^{-1}), peroxidase (POD, µmol mg^{-1} protein min^{-1}), ascorbate peroxidase (APX, µmol mg^{-1} protein min^{-1}) and concentration of malondialdehyde (MDA, nmol g^{-1} FW) and hydrogen peroxide (H_2O_2, µmol g^{-1} FW) in the leaves of pistachio plants grown for seven weeks under saline conditions without or with the application of Si. The data are the mean ± SD of four replicates. The data of each column indicated by the same letter are not significantly different ($P < 0.05$).

Treatments		SOD	CAT	POD	APX	H_2O_2	MDA
−Si	Control	23.3 ± 2.25 ^{c}	58.7 ± 6.54 ^{b}	0.27 ± 0.04 ^{b}	0.76 ± 0.05 ^{a}	0.44 ± 0.08 ^{b}	13.3 ± 2.61 ^{b}
	Salinity	33.2 ± 3.07 ^{b}	79.7 ± 7.11 ^{a}	0.49 ± 0.05 ^{a}	1.02 ± 0.07 ^{ab}	1.03 ± 0.07 ^{a}	30.4 ± 5.53 ^{a}
+Si	Control	23.2 ± 2.47 ^{c}	57.4 ± 8.39 ^{b}	0.28 ± 0.04 ^{b}	0.78 ± 0.05 ^{a}	0.46 ± 0.08 ^{b}	14.4 ± 2.98 ^{b}
	Salinity	40.9 ± 2.64 ^{a}	89.2 ± 7.61 ^{a}	0.52 ± 0.08 ^{a}	1.08 ± 0.09 ^{a}	1.01 ± 0.03 ^{a}	15.9 ± 2.74 ^{b}

Discussion

Pistachio is a salt-tolerant glycophyte species and its growth is not affected by lower salinity levels (4 dS m^{-1}). A significant reduction of dry weight starts at a salinity of 8 dS m^{-1} (33). In this work, the applied salt level was higher than other reports of pistachio plants in order to evaluate the effect of Si under severe salinity in this tolerant species.

Si application improved the dry matter production of pistachio plants as compared to those without Si supply under salinity conditions. The obtained results are in agreement with findings on Si-accumulators, such as rice, wheat and sorghum (18, 34), and non-accumulators such as tomato (15).

The maximum quantum yield of PSII (F_v/F_m) is an indication of overall photosynthetic capacity (35). In addition, the non-photochemical quenching (qN) of PSII, as an indicator of the heat dissipation process, plays a key role in the protection of PSII against excess excitation energy that is produced under various stresses (36). A significant reduction of F_v/F_m in salt-stressed pistachio indicated that a proportion of the PSII reaction centres are damaged or inactivated following photoinhibition, commonly observed in plants under stress (37). Photoinhibition has been defined as the inhibition of photosynthesis caused by excessive radiation energy (38). A simultaneous increase of qN in salt-stressed pistachio may help plants against damage induced by excess excitation energy (37). Silicon application ameliorated reduction of electron transport capacity and F_v/F_m ratio in salt-stressed pistachio plants. Information on the effect of Si on leaves' photochemical parameters is rare and restricted to Si-accumulator Gramineae species (24). Our data confirmed the effect of Si application on the protection of the photochemical process in pistachio as a non-accumulator species.

The inhibition of growth under salinity is often ascribed to a reduction of plants' photosynthetic performance (5). Surprisingly, salt stress caused a significant reduction of the photosynthetic rate (*A*) in pistachio. The enrichment of saline soil with Si resulted in a significant increase of photosynthesis when compared to salt-stressed plants grown without Si. It has previously been reported that exogenously applied Si improved the net CO_2 assimilation rate in Si-accumulators, such as maize (25), and non-accumulators, such as tomato (15). The application of Si also elevated water-use efficiency (*WUE*) in salt-stressed plants. The improvement of the *WUE* upon Si application was the consequence of higher CO_2 fixation along with stable amounts of transpiration in Si-treated plants.

One of the mechanisms for the Si-mediated protection of salt-stressed plants is the reduction of Na and the enhancement of K uptake (13). In salt-stressed pistachio, Si lowered the Na concentration in both the leaves and the roots, while the K concentration remained stable in the leaves and increased in the roots. In the absence of salt, however, a higher root K concentration was associated with lower K in the leaves in the Si-treated plants. It probably indicated that the root-shoot transport of K was somewhat reduced by Si. The mechanism for this effect is obscure.

The effect of Si on Na partitioning into CW-bound and soluble cell fractions has been studied in Si-accumulator wheat plants (11). In that report, it was speculated that Si application causes Na detoxification via increasing CW-bound Na (11). We observed here, however, that Si treatment did not affect concentration of CW-bound Na, while it decreased that of free Na in the leaves. It has been reported that the deposition of Si in the cell walls of roots decreases the translocation of salts to the leaves. In the present study, a reduction in foliar Na^+ content was obtained when salinized pistachio plants were treated with Si. Thus, our results support the hypothesis that the alleviation of the deleterious effects of salt by Si in pistachio can be related to the reduction of salt accumulation in the leaves. This mechanism may be considered to be another reason for the ameliorative effect of Si in salt-stressed pistachio. In addition, a lack of any change in the CW-bound Na by Si in pistachio - in contrast to wheat - may reflect the well-known difference in the CW structure between monocotyledonous and dicotyledonous species (39).

Salt stress induces overproduction of ROSs, which trigger phytotoxic reactions such as lipid peroxidation, protein degradation and DNA damage (4). Increased lipid peroxidation following oxidative damage under salinity has been reported in several plant species (3, 7). In the present study, despite the expected enhancement in the activity of all four studied antioxidative enzymes under salt stress, Si application caused significant stimulation only in the activity of SOD. Similar results on the activation of the antioxidative capacity of plants by Si have been reported by other authors in Si-accumulator (34) and non-accumulator (15, 21) species. Therefore, Si improved cellular membrane stability through the stimulation of SOD activity because the MDA concentration as an indicator of lipid peroxidation was significantly lower in Si-applied pistachio under salt stress. However, it is important to note that the balance between SODs and the different H_2O_2-scavenging enzymes in cells is considered to be crucial in determining the steady-state level of H_2O_2, but the alternative antioxidant

enzymes, such as glutathione reductase and glutathione peroxidase, are important enzymes in protecting against oxidants under stress conditions. Therefore, the possible mechanisms of the Si-mediated improvement of the antioxidative capacity in salt-stressed pistachio may be attributed to the activity of these alternative antioxidant enzymes (although we did not measure their activities). Furthermore, ROSs are part of a complex metabolic regulatory network which cannot be understood by measuring global enzyme activities (which represent the activity of different compartments). For example, several studies have shown that a protective role of Si against the oxidative stress in higher plants coincided with reduced SOD, CAT and POD activity and decreased lipid peroxidation (40).

In addition, a higher leaf Ca concentration in salt-stressed pistachio may be another mechanism for higher membrane integrity in Si-treated plants. Calcium plays a vital role in maintaining membrane stability and permeability (41).

Conclusion

High soil salinity had an adverse effect on pistachio plants while exogenous Si alleviated the effects of salt on plant growth. Various mechanisms were involved in the Si-mediated alleviation of salt stress in pistachio. A higher CO_2 fixation rate and *WUE* and an elevated level of SOD activity, a reduction of the total Na concentration, and a decline in Na partitioning into cytosolic fraction, were all observed in Si-supplemented plants in this work. In addition, the maintenance of membrane integrity as the consequence of activated antioxidative defence and a higher leaf Ca concentration was observed in Si-supplemented pistachio plants under saline conditions.

REFERENCES

1. Ashraf, M. (2009). Biotechnological approach of improving plant salt tolerance using antioxidants as markers. *Biotechnol. Adv.*, 27, 84-93.
2. Saleem, A., Ashraf, M. and Akram, N.A. (2011). Salt (NaCl)-induced modulation in some key physio-biochemical attributes in okra (*Abelmoschus esculentus* L.). *J. Agron. Crop Sci*., 197, 202-213.
3. Munns, R. and Tester, M. (2008). Mechanisms of salinity tolerance. *Annu. Rev. Plant Biol*., 59, 651-681.
4. Hasanuzzaman, M., Hossain, M.A. and Fujita, M. (2011). Selenium-induced up-regulation of the antioxidant defense and methylglyoxal detoxi fi cation system reduces salinity-induced damage in rapeseed seedlings. *Biol. Trace Elem. Res*., 143, 1704-1721.
5. Ahmed, M., Hassen, F.U., Qadeer, U., Aslam, M.A. (2011). Silicon application and drought tolerance mechanism of sorghum. *Afr. J. Agric. Res.*, 6, 594-607.
6. Shahbaz, M. and Ashraf, M. (2007). Influence of exogenous application of brassinosteroid on growth and mineral nutrients of wheat (*Triticum aestivum* L.) under saline conditions. *Pak. J. Bot*., 39, 513-522.
7. Miller, G., Suzuki, N., Ciftci-Yilmaz, S. and Mittler, R. (2010). Reactive oxygen species homeostasis and signaling during drought and salinity stresses. *Plant Cell Environ*., 33, 453-467.
8. Dat, J., Vandenabeele, S., Vranova´, E., van Montagu, M., Inze´, D. and van Breusegem, F. (2000). Dual action of active oxygen species during plant stress responses. *Cell Mol. Life Sci*., 57, 779-795.
9. Ghahremani, M., Ghanati, F., Bernard, F., Gholami, M. and Azad, T. (2013). Effects of exogenous ornithine enantiomers on tobacco cells under salinity conditions. *Prog. boil. Sci*., 3, 100-107.
10. Ding, M., Hou, P. and Shen, X. (2010). Salt-induced expression of genes related to Na^+/K^+ and ROS homeostasis in leaves of salt-resistant and salt-sensitive poplar species. *Plant Mol. Biol*., 73, 251-269.
11. Saqib, M., Zörb, C. and Schubert, S. (2008). Silicon-mediated improvement in the salt resistance of wheat (*Triticum aestivum*) results from increased sodium exclusion and resistance to oxidative stress. *Func. Plant Biol.*, 35, 633-639.
12. Epstein, E. (2009). Silicon: its manifold roles in plants. *Ann. Appl. Biol*., 155, 155-160.
13. Liang, Y., Zhang, W., Chen, Q. and Ding, R. (2005). Effects of silicon on H^+-ATPase and H^+-PPase activity, fatty acid composition and fluidity of tonoplast vesicles from roots of salt-stressed barley (*Hordeum vulgare* L.). *Environ. Exp. Bot*., 53, 29-37.
14. Tuna, A.L, Kaya, C., Higgs, D., Murillo-Amador, B., Aydemir, S. and Gergon, A.R. (2008). Silicon improves salinity tolerance in wheat plants. *Environ. Exp. Bot*., 62, 10-16.
15. Al-Aghabary, K., Zhu, Z. and Shi, Q. (2004). Influence of silicon supply on chlorophyll content, chlorophyll fluorescence and antioxidative enzyme activities in tomato plants under salt stress. *J. Plant Nutr*., 27, 2101-2115.

16. Hashemi, A., Abdolzadeh, A. and Sadeghipour, H.R. (2010). Beneficial effects of silicon nutrition in alleviating salinity stress in hydroponically grown canola, *Brassica napus* L., plants. *Soil Sci. Plant Nutr*., 56, 244-253.

17. Gunes, A., Inal, A., Bagei, E.G. and Pilbeam, D.J. (2007). Silicon-mediated changes of some physiological and enzymatic parameters symptomatic for oxidative stress in spinach and tomato grown in sodic-B toxic soil. *Plant and Soil*, 290, 103-114.

18. Sonobe, K., Hattori, T., An, P., Tsuji, W., Eneji, A.E., Kobayashi, S., Kawamura, Y., Tanaka, K. and Inanaga, S. (2011). Effect of silicon application on sorghum root responses to water stress. *J. Plant Nutr*., 34, 71-82.

19. Cooke, J. and Leishman, M.R. (2011). Is plant ecology more siliceous than we realise? *Trends Plant. Sci*., 16, 61-68.

20. Yin, L., Wang, S., Li, J., Tanaka, K. and Oka, M. (2013). Application of silicon improves salt tolerance through ameliorating osmotic and ionic stresses in the seedling of *Sorghum bicolor*. *Acta Physiol. Plant.,* 35, 3099-3107.

21. Tahir, M.A., Aziz, T., Farooq, M. and Sarwar, G. (2012) Silicon-induced changes in growth, ionic composition, water relations, chlorophyll contents and membrane permeability in two salt-stressed wheat genotypes. *Arch. Agron. Soil Sci*., 58, 247-256.

22. Gijón, M.C., Gimenez, C., Perez-López, D., Guerrero, J., Couceiro, J.F. and Moriana, A. (2011). Water relations of pistachio (*Pistacia vera* L.) as affected by phenological stages and water regimes. *Sci. Hort*., 128, 415-422.

23. Sheibani, A. (1994). Pistachio production in Iran. *Acta Hort*., 419, 14-15.

24. Habibi, G. and Hajiboland, R. (2013). Alleviation of drought stress by silicon supplementation in pistachio (*Pistacia vera* L.) plants. *Folia Hort*., 25, 21-29.

25. Parveen, N. and Ashraf, M. (2010). Role silicon in mitigating the adverse effects of salt stress on gowth and phytosynthetic attributes of two maize (*Zea mays* L.) cultivars grown hydroponically. *Pak. J. Bot.*, 42, 1675-1684.

26. Hajiboland, R., Aliasgharzadeh, N., Laiegh, S.F. and Poschenrieder, C. (2010). Colonization with arbuscular mycorrhizal fungi improves salinity tolerance of tomato (*Solanum lycopersicum* L.) plants. *Plant Soil*, 331, 313-327.

27. Krall, J.P. and Edwards, G.E. (1992). Relationship between photosystem II activity and CO_2 fixation in leaves. *Physiol. Plant*., 86, 180-187.

28. Habibi, G. and Hajiboland, R. (2012). Comparison of photosynthesis and antioxidative protection in *Sedum album* and *Sedum stoloniferum* (Crassulaceae) under water stress. *Photosynthetica*, 50, 508-518.

29. Bradford, M.M. (1976). A rapid and sensitive method for quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.*, 72, 248-254.

30. Velikova, V., Yordanov, I. and Edreva, A. (2000). Oxidative stress and some antioxidant systems in Acid rain-treated bean plants-protective role of exogenous polyamines. *Plant Sci*., 151, 59-66.

31. Jaiswal, P.C. (2004) Soil, Plant and Water Analysis. New Delhi, Kalyani Publishers.

32. Rogalla, H. and Römheld, V. (2002). Role of leaf apoplast in silicon-mediated manganese tolerance of *Cucumis sativus* L. *Plant Cell Environ*., 25, 549-555.

33. Banakar, M.H. and Ranjbar, G.H. (2010). Evaluation of Salt Tolerance of Pistachio Cultivars at Seedling Stage. *American-Eurasian J. Agric. Environ. Sci*., 9, 115-120.

34. Ávila, F.W., Baliza, D.P., Faquin, V., Araujo, J. and Ramos, S.J. (2010). Silicon-nitrogen interaction in rice cultivated under nutrient solution. *Rev. Cienc. Agron*., 41, 184-190.

35. Balouchi, H.R. (2010). Screening wheat parents of mapping population for heat and drought tolerance, detection of wheat genetic variation. *Int. J. Biol. Life Sci*., 6, 56-66.

36. Ruban, A.V., Pascal, A.A., Robert, B. and Horton, P. (2002). Activation of zeaxanthin is an obligatory event in the regulation of photosynthetic light harvesting. *J. Biol. Chem*., 277, 7785-7789.

37. Vaz, J. and Sharma, P.K. (2011). Relationship between xanthophy cycle and non-photochemical quenching in rice (*Oryza sativa* L.) plants in response to light stress. *Indian J. Exp. Bot*., 49, 60-67.

38. Baker, N.R. and Rosenqvist, E. (2004). Applications of chlorophyll fluorescence can improve crop production strategies: an examination of future possibilities. *J. Exp. Bot*., 55, 1607-1621.

39. Broadley, M., Brown, P., Cakmak, I., Ma, J.F., Rengel, Z. and Zhao, F. (2012). Beneficial elements. In Marschner,P. (ed.), *Marschner's Mineral Nutrition of Higher Plants*, 3rd Ed., Elsevier, Oxford, UK, pp. 249-269.

40. Miao, B.H., Han, X.G. and Zhang, W.H. (2010). The ameliorative effect of silicon on soybean seedlings grown in potassium-deficient medium. *Ann. Bot.*, 105, 967-973.

41. Hawkesford, M., Horst, W., Kichey, T., Lambers, H., Schjoerring, J., Skrumsager Møller, I. and White, P. (2012). Function of macronutrients. In Marschner,P. (ed.), *Marschner's Mineral Nutrition of Higher Plants*, 3rd Ed., Elsevier, Oxford, UK, pp. 135-189.