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Effects of silicon supplementation on wheat plants under salt stress

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Abstract:

Silicon (Si) is the second most abundant element in the earth's crust and is regarded as a beneficial element for higher plants. In this study, the effect of Si supplementation (1 and 4 mM) was studied on wheat (*Triticum aestivum* cv. Homa) plants grown hydroponically under salinity stress (50 and 150 mM NaCl) for two weeks under controlled environmental conditions. Plant biomass was found to decrease at both salinity levels. Contrary to our expectation, Si supplementation failed to mitigate the salt stress effects on dry matter production. However, the majority of biochemical parameters related to salt tolerance showed improvements as a result of Si application, particularly at 1 mM. Under both control and salinity conditions, Si treatment resulted in higher leaf contents of chlorophyll and carotenoids, soluble sugars, proteins, and free amino acids, particularly proline. Osmotic potential, however, declined in the roots, suggesting that Si supplementation might contribute to plant's higher water uptake capacity. Si treatment diminished leaf concentration of Na^+ in the cell sap but increased it in the cell wall-bound fraction, indicating a Na^+ detoxification mechanism mediated by Si. Our results suggest that a short-term Si treatment affects biochemical indicators of salt tolerance but that long-term exposure to Si is needed for a significantly alleviating effect on plant biomass.

Keywords: Cell-wall Na^+ binding, Osmotic potential, Proline, Soluble sugars.

Introduction:

Salinity is one of the most stressful factors that limits plant growth and productivity, particularly in arid and semi-arid regions of the world (Munns and Tester, 2008). Salt stress comprises the two main components of osmotic stress and ion toxic effects. Osmotic effects could be recognized by water relations while ionic effects of salt could be studied via leaf analysis to detect Na^+ accumulation (Hasegawa *et al.*, 2000). Plant's ability to maintain growth under lower salt concentrations is based on mechanisms providing osmotic homeostasis while growth under higher salt concentrations is mainly induced by ion homeostasis mechanisms (Hasegawa *et al.*, 2000).

Silicon (Si) is the second most abundant element in soil. Plants are classified into Si-accumulator and non-accumulator species based on their capacity for Si uptake and accumulation (Broadley *et al.*, 2012). Although Si has not yet been categorized as an essential nutrient for higher plants, it has been demonstrated to be beneficial for the growth of plants, especially those belonging to the family Gramineae (Broadley *et al.*, 2012; Hajiboland, 2012). Si in the growth medium alleviates the effects of various environmental stresses such as salinity, drought, chilling, and freezing or those of UV radiation and heavy metal toxicity (Currie and Perry, 2007; Liang *et al.*, 2007; Hajiboland, 2012; Zhu and Gong, 2014). A number of studies have been dedicated to Si functions in alleviating salt stress effects in Si-accumulator species such as rice (Yeo *et al.*, 1999; Gong *et al.*, 2006), barley (Liang 1999; Liang *et al.*, 2007), zucchini (Savvas *et al.*, 2009), and cucumber (Zhu *et al.*, 2004) as well as in some non-accumulator species such as tomato (Al-Aghabary *et al.*, 2004; Romero-Aranda *et al.*, 2006), canola (Hashemi *et al.*, 2010), and tobacco (Hajiboland and Cheraghvareh, 2014).

Various mechanisms have been claimed to be involved in such ameliorative effects of Si under salt stress as activation of antioxidative defense and improvement of plant's ability to uptake water and nutrients from the soil (Currie and Perry, 2007; Liang *et al.*, 2007; Hajiboland, 2012; Zhu and Gong, 2014). Under salt stress, Si reportedly reduces Na^+ uptake but increases its K^+:Na^+ ratio, alleviating the ion toxicity effect in several plant species such as rice (Yeo *et al.*, 1999) and barley (Liang, 1999). Another possible mechanism for Si-mediated amelioration of salt stress comes from the finding that Si-mediated Mn^{2+} (Rogalla and Römheld, 2002) and Al^{3+} (Wang *et al.*, 2004) toxicities are the consequence of binding these metals to the cell wall. In the presence of Si, Mn^{2+} is bound more strongly to the cell wall to decrease Mn^{2+} concentration in the symplast (Rogalla and Römheld, 2002). Detoxification of Al^{3+} in the apoplast of the root apex and its reduced mobility are achieved by forming

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hydroxyl aluminum silicates (Wang *et al.*, 2004). Saqib *et al.* (2008) demonstrated that Si-mediated salt stress amelioration depends not only on reducing Na^+ uptake and shoot-root transport but also on the increased cell wall Na^+ binding and the reduced cell sap Na^+ concentration.

Wheat is a moderately salt resistance crop and an Si-accumulating species (Broadley *et al.*, 2012). Alleviation of salt stress has been observed as a result of Si application in hydroponics (Saqib *et al.*, 2008; Tuna *et al.*, 2008) and its application to soil (Tahir *et al.*, 2006; Ali *et al.*, 2012). It has been demonstrated that Si decreases Na^+ concentrations in the leaves and roots of wheat, leading to its enhanced salt resistance (Tahir *et al.*, 2006; Saqib *et al.*, 2008; Tuna *et al.*, 2008; Ali *et al.*, 2012). In addition, the evidence provided by Saqib *et al.* (2008) indicates a role for cell wall Na^+ binding in the Si-mediated growth amelioration in salt-stressed wheat. Wheat has a high capacity for Si uptake and accumulation (Broadley *et al.*, 2012); it will, therefore, be interesting to know the effects of higher Si concentrations on the growth and salt stress alleviation in this species. The different levels of contributions made by the mechanisms providing osmotic and ion homeostases under mild and severe salinity (Hasegawa *et al.*, 2000) warrants a detailed analysis of Si effects under two contrastive levels of salt.

This study was conducted to compare the effects of different Si concentrations on the amelioration of salt stress with regard to the osmotic and ion toxicity components of salt. In addition, Na^+ binding to the cell wall and its contribution to the Si-mediated amelioration of salinity were also evaluated under mild and serve salinity stresses.

Materials and Methods:

Plant culture and treatments: Seeds of wheat plants (*Triticum aestivum* L. cv. Homa) provided by Dryland Agricultural Research Institute (DARI) (Maragheh, Iran) were surface-sterilized using sodium hypochlorite at 5% and allowed to germinate in the dark on perlite. Seven-day-old young seedlings were transferred to 2 L pots (9 plants per pot) containing the Hoagland (Hoagland and Arnon, 1950) nutrient solution (pH 5.8) and precultured for further 3 days. After neutralization by HCl, Si (as Na_2SiO_3) was added at the three levels of 0, 1, and 4 mM (final concentration in the nutrient solution). Three days after Si application, salinity treatments were started at the following three levels of NaCl: control (0 mM), low (50 mM), and high (150 mM). Nutrient solutions were replaced weekly. Plants were grown under controlled environmental conditions maintaining a temperature regime of 25 °/18 °C day/night, 14/10 h light/dark period, a relative humidity of 70/80%, and at a photon flux density of about 300 $\mu mol\ m^{-2}s^{-1}$.

Plants harvest, growth parameters, leaf pigments, and osmotic potential: The plants were harvested two weeks after exposure to the different experimental salinity conditions when their shoots and roots were separated. The plants were then allowed at 70 °C for 2 d before they were measured for their fresh and dry weights.

Leaf concentration of chlorophyll (Chl *a, b)* and carotenoids (Car) were determined according to Lichtenthaler and Wellburn (1985). Briefly, the leaves were homogenized in 80% cold acetone in the dark at 4 °C. After 24 h, sample absorptions were determined at 663 (Chl *a*), 646 (Chl *b*), and 470 (Car) nm using a spectrophotometer (Specord 200, Analytic Jena, Jena, Germany). Determination of anthocyanins was performed using a pH differential method at pH 1 and pH 4.5 in the methanol/HCl (98:2, v/v) extract and reported as mg of cyanidine-3-glucoside g^{-1} FW (Giusti and Wrolstad, 2001). Total flavonoid content was determined in the methanol extract of leaves. An aliquot of 1 ml of the solution containing 1 mg of the extracts in methanol was added to test tubes containing 0.1 ml of 10% Al $(NO_3)_3$, 0.1 ml of 1 M potassium acetate, and 3.8 ml of methanol. After 40 min at room temperature, absorbance was recorded using a spectrophotometer at 415 nm. Quercetin was used as the standard (Grayer, 1989).

Leaf and root osmotic potentials were determined in plants harvested 1 h after the lights were turned on. The leaf and root samples were homogenized in a prechilled mortar and pestle and centrifuged at 4000 g for 20 min at 4 ºC. The osmotic pressure of the samples was measured using an osmometer (Micro-Osmometer, Heman Roebling Messtechnik, Germany) and the miliosmol data were recalculated to MPa.

Determination of organic osmolytes and soluble proteins*:* To determine non-structural carbohydrates, the samples were homogenized in 100 mM phosphate buffer (pH 7.5) at 4 ^oC after centrifugation at 12000 g for 15 min. The supernatant thus obtained was used to determine total soluble sugars whereas the pellets were kept for starch analysis according to the method described in Yemm and Willism (1954). An aliquot of the supernatant was mixed with anthrone–sulfuric acid reagent and incubated for 10 min at 100 $^{\circ}C$. Upon cooling, absorbance was determined at 625 nm. The standard curve was created using glucose (Merck). To determine the starch content, the pellet was resuspended in a 4:1 (v/v) mixture of 8 N HCl/dimethylsulfoxide (Merck) to dissolve the starch by agitating for 30 min at 60 ^oC. After centrifugation, the supernatant was mixed with the iodine–HCl solution and left for 15 min at room temperature before absorbance was determined at 600 nm. Starch (Merck) was used for the generation of the standard curve.

To determine the soluble proteins and total free α-amino acids, the samples were homogenized in 100 mM phosphate buffer (pH 6.5) and centrifuged at 12000 g for 15 min. The supernatant was used for the determination of total soluble proteins using a commercial reagent (Bradford reagent, Sigma) and bovine albumin serum as the standard. Free amino acid

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concentrations were assayed using the ninhydrin colorimetric method and glycine was employed for creating the standard curve (Yemm and Cocking, 1955). Proline was extracted and determined by the method of Bates *et al.* (1973). Leaf tissues were homogenized with 3% sulfosalicylic acid and the homogenate was centrifuged at 3000 g for 20 min. The supernatant was treated with acetic and ninhydrin acids before it was boiled for 1 h. Absorbance was determined at 520 nm. Proline (Sigma) was used to generate the standard curve.

Determination of Na^+: Leaves and roots of each replicate were divided into two identical subsamples and used for parallel determination of total and cell sap Na^+ concentrations. One of the two subsamples was directly oven-dried, and transferred to porcelain crucibles where it was dry-ashed at 550 ºC for 8 h. The samples thus obtained were resolved in 0.5 M HCl and made up to volume with double-distilled water. The other subsample was used for the extraction of cell sap according to the procedure described in Saqib *et al.* (2008). The fresh samples were homogenized in a tube using a glass rod. The homogenate was then centrifuged at 2000 g for 5 min at 4 ºC and the supernatant thus obtained was directly used to analyze the cell sap Na^+ content after dilution with distilled water. Cell wall-bound Na^+ was calculated as the difference between the total Na^+ and the amount of Na^+ in the sap fraction. Na^+ concentration was determined using a flame photometer (Jenway, PFP7).

Experimental design and data analysis: This experiment was conducted in a randomized block design with four replications as four independent pots and two factors including salinity and Si application. Differences between the means were detected via the Tukey test ($P<0.05$) using Sigma Stat 2.03.

Results:

Shoot dry weight (DW) decreased with both salinity levels tested in the presence and absence of Si. A significant reduction was, however, observed in Root DW only with +Si plants. Si supplementation resulted in higher shoot and root biomass in the control plants while no such effect was observed in the salt-treated ones (Fig. 1).

Salt treatment decreased leaf concentrations of Chl *a*, *b*, and Car in both –Si and +Si plants. However, these parameters increased in both the control and the salt-treated plants as a result treatment with 1 mM Si but treatment with higher levels of Si (4 mM)again decreased the concentrations of leaf pigments in both control and salt-treated plants. In contrast to the Chl and Car, anthocyanins concentration was significantly higher in the leaves of salt-treated plants and Si treatment further increased it. Thisis while leaf flavonoids content was not influenced by salt treatment. Supplementation of plants with Si led to reduced leaf flavonoids; the reductions observed were significant in the control plants at both Si levels but at higher Si level in the salt-treated ones (Table 1).

Leaf and root osmotic potentials were expectedly lower in the salt-treated plants. In the leaves of the plants treated with a low Si level (1 mM), osmotic potential was less negative than that in the –Si plants. This effect was significant in the absence of salt treatments. The roots, in contrast, recorded lower osmotic potentials in the Si-treated plants. This effect was significant in the control plants in response to 1 mM of Si and in salt-treated plants in response to 4 mM of Si (Fig. 2).

Concentration of soluble proteins declined in both leaves and roots as a result of salt treatments. Si supplementation increased the concentration of soluble proteins in both control and salt-treated plants. This effect was more prominent under lower (1 mM) than higher levels (4 mM) of Si treatment. Similar effects of salt were observed in the case of leaf and root free amino acids. The effectd of Si on free amino acids, however, was much less expressed than that observed with leaf soluble proteins while Si had no such effect on root amino acid concentration (Table 2).

In the absence of Si, leaf proline concentration increased under a low (50 mM) salt concentration but decreased when it rose to 150 mM. Under the same conditions, leaf proline increased with increasing salinity, with the highest recorded in the leaves under a salt concentration of 150 mM. The 4 mM Si treatment resulted in higher proline concentrations in the leaves of both control and salt-treated plants (Fig. 3). Unlike the leaves, root proline concentrations were higher in the both +Si and –Si salt-treated plantsplants. Finally, plants treated with a high Si level recorded even lower proline concentrations in their roots (Fig. 3).

Leaf and root soluble sugars recorded increased concentrations in response to salt treatment. Low (rather than high) Si levels further increased their concentrations under both control and low salinity conditions. Higher Si levels led to an increase in soluble sugar concentrations but only in the absence of salt treatment. Similar trends were observed in both leaf and root starch contents in response to salinity. Treatment with low levels of Si was found to increased starch concentrations in leaves while high Si levels decreased it. In contrast to leaves, roots exhibited only slightly increased starch concentrations in the Si-supplemented plants although no significant differences were observed in this parameter under the three levels of Si applied (Table 2).

As expected, total and cell sap Na^+ concentrations increased with increasing salinity in both leaves and roots. Similarly, cell wall-bound fraction of Na^+ was higher under salt treatments. However, differences were observed only in plants treated with high (4 mM) level of Si between the two salt treatments, with significant levels of both Na^+ concentrations obtained with the high salinity treatment. Cell wall-bound Na^+ concentration, however, reduced as a result of Si treatments under control and low salt treatment while it increased in i

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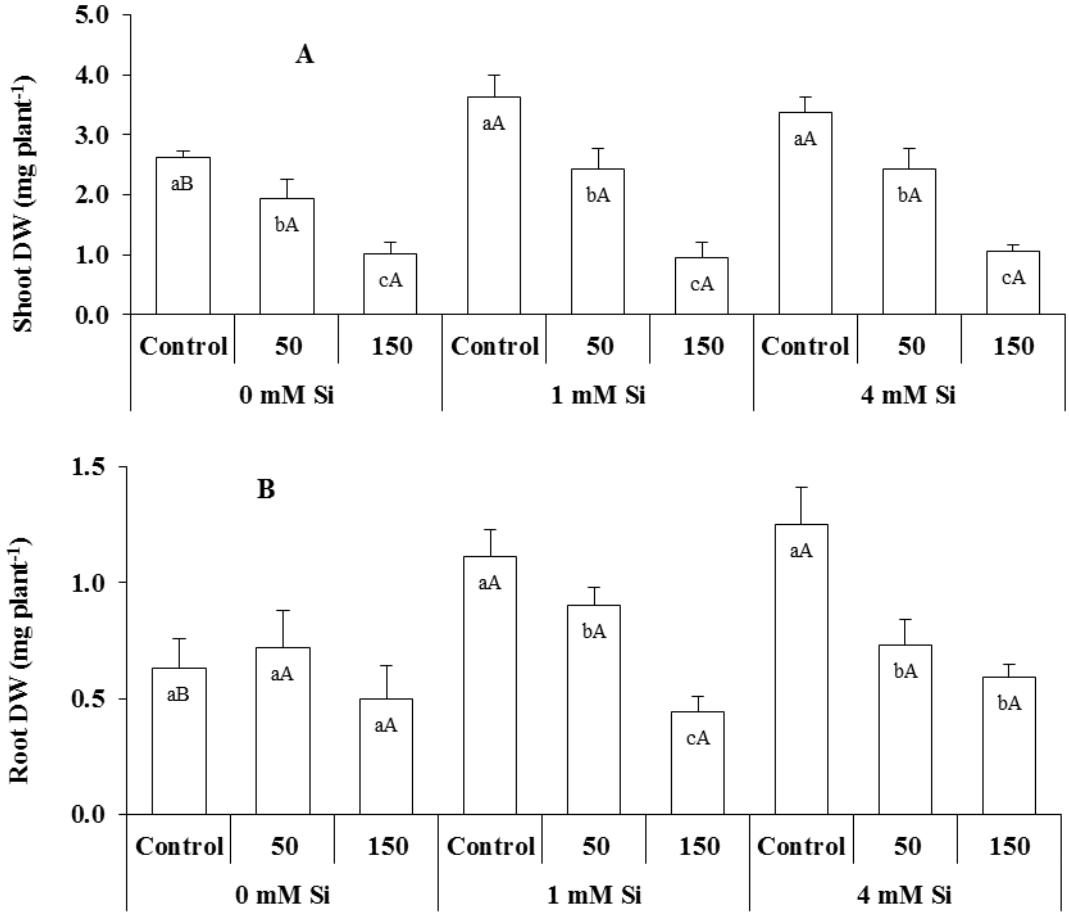


Figure 1. Dry weight (DW) of shoots (A) and roots (B) in wheat (*Triticum aestivum* L. cv. Homa) plants grown hydroponically under three levels of NaCl salinity (control, 50, and 150 mM) and Si (as Na_2SiO_3) for two weeks. Significant differences among the three NaCl levels within each Si treatment are indicated by different lower case letters while those between different Si levels within each NaCl treatment are indicated by different uppercase letters (P<0.05).

Table 1. Concentrations (mg g^{-1} FW) of leaf pigments in wheat (*Triticum aestivum* L. cv. Homa) plants grown hydroponically under three levels of NaCl salinity (control, 50, and 150 mM) and Si (as Na_2SiO_3) for two weeks.

Treatment		Chl *a*	Chl *b*	Carotenoids	Anthocyanins	Flavonoids
Si	NaCl					
0 mM	Control	0.50±0.04 ^{aB}	0.14±0.02 ^{aB}	0.17±0.01 ^{aC}	2.70±0.29 ^{cB}	0.39±0.01 ^{aA}
	50	0.42±0.03 ^{abB}	0.10±0.02 ^{abA}	0.13±0.01 ^{bB}	7.21±0.35 ^{bA}	0.38±0.01 ^{aA}
	150	0.32±0.05 ^{bB}	0.08±0.00 ^{bA}	0.11±0.01 ^{cC}	10.2±1.02 ^{aA}	0.35±0.01 ^{aA}
1 mM	Control	0.86±0.06 ^{aA}	0.35±0.01 ^{aA}	0.25±0.00 ^{aA}	3.67±1.21 ^{cAB}	0.35±0.01 ^{aB}
	50	0.61±0.04 ^{bA}	0.11±0.03 ^{bA}	0.17±0.02 ^{bA}	8.11±1.46 ^{bA}	0.37±0.01 ^{aA}
	150	0.65±0.01 ^{bA}	0.09±0.02 ^{bA}	0.16±0.01 ^{bA}	12.7±1.41 ^{aA}	0.34±0.01 ^{aA}
4 mM	Control	0.74±0.08 ^{aA}	0.08±0.01 ^{aC}	0.23±0.00 ^{aB}	5.04±1.53 ^{aA}	0.32±0.01 ^{aC}
	50	0.42±0.03 ^{bB}	0.08±0.02 ^{aA}	0.15±0.02 ^{bAB}	6.67±1.88 ^{aA}	0.33±0.01 ^{aB}
	150	0.40±0.06 ^{bB}	0.01±0.00 ^{bB}	0.14±0.01 ^{bB}	9.68±0.18 ^{aA}	0.31±0.01 ^{aB}

In each column, significant differences among the three NaCl levels within each Si treatment are indicated by different lower case letters and those between different Si levels within each NaCl treatment are indicated by different uppercase letters (P<0.05).

plants treated with a high salt concentration and an Sconcentration of 4 mM (Fig. 4).

Like leaf Na^+ concentrations, total and cell sap Na^+ concentrations increased under salt treatments while Si supplementation increased it even further in the control plants not subjected to the salt treatment. Cell wall bound Na^+, however, decreased as a result of Si supplementation and no differences were observed in this regard between the two levels of Si (Fig. 5).

Discussion:

Effects of Si on the amelioration of salt stress: Contrary to our expectations, Si supplementation had an only slight influence on the growth of salt-stressed wheat plants. Contrary to our findings, other reports have shown significant effects of Si addition on the

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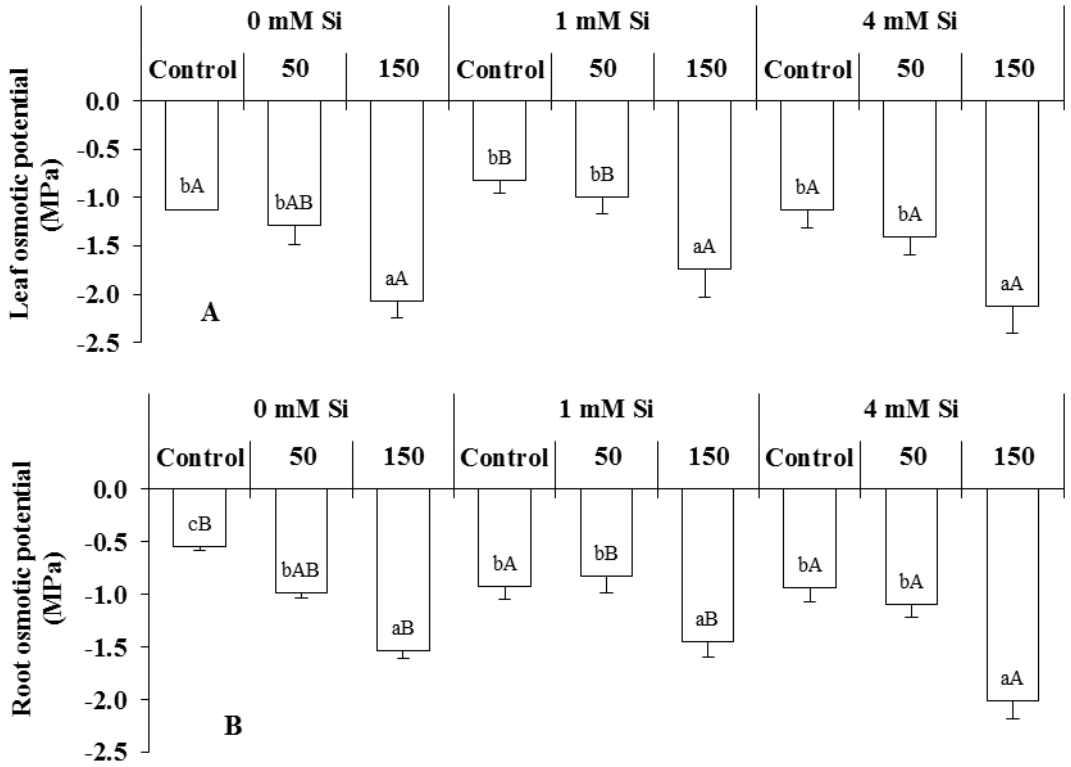


Figure 2. Osmotic potentials (MPa) of leaves (A) and roots (B) of wheat (*Triticum aestivum* L. cv. Homa) plants grown hydroponically under three levels of NaCl salinity (control, 50, and 150 mM) and Si (as Na_2SiO_3) for two weeks. Significant differences among the three NaCl levels within each Si treatment are indicated by different lower case letters and those between different Si levels within each NaCl treatment are indicated by different uppercase letters (P<0.05).

Table 2. Concentrations of soluble proteins (mg g^{-1} FW), total free amino acids (µmol g^{-1} FW), soluble sugars, and starch (mg g^{-1} FW) in the leaves and roots of wheat (*Triticum aestivum* L. cv. Homa) plants grown hydroponically under three levels of NaCl salinity (control, 50, and 150 mM) and Si (as Na_2SiO_3) for two weeks.

Treatment		Soluble proteins		Free amino acids	
Si	NaCl	Leaves	Roots	Leaves	Roots
0 mM	Control	7.78±0.49 ^{aB}	4.41±0.08 ^{aC}	27.8±0.55 ^{aB}	13.7±0.18 ^{aA}
	50	6.05±0.51 ^{bB}	3.35±0.11 ^{bB}	20.8±0.26 ^{bC}	12.2±0.37 ^{bA}
	150	4.14±0.34 ^{cC}	2.18±0.44 ^{cB}	19.7±0.74 ^{cB}	10.8±0.63 ^{cA}
1 mM	Control	11.9±1.82 ^{aA}	5.48±0.29 ^{aB}	29.7±0.97 ^{aA}	14.1±0.52 ^{aA}
	50	8.68±0.19 ^{bA}	4.47±0.32 ^{bA}	26.3±0.43 ^{bA}	12.4±0.24 ^{bA}
	150	5.67±0.18 ^{cA}	3.14±0.52 ^{cA}	25.8±1.51 ^{bA}	11.6±0.25 ^{cA}
4 mM	Control	8.64±0.54 ^{aB}	5.98±0.23 ^{aA}	28.8±0.46 ^{aAB}	14.3±0.17 ^{aA}
	50	6.86±0.49 ^{bB}	4.42±0.39 ^{bA}	23.8±0.92 ^{bB}	12.8±0.85 ^{bA}
	150	5.05±0.30 ^{cB}	3.77±0.09 ^{cA}	20.6±1.34 ^{cB}	11.3±0.41 ^{cA}
		Soluble sugars		Starch	
		Leaves	Roots	Leaves	Roots
0 mM	Control	22.8±0.98 ^{cC}	12.3±1.20 ^{cB}	0.66±0.04 ^{cB}	1.16±0.04 ^{cA}
	50	28.1±1.17 ^{bB}	22.3±1.25 ^{bB}	1.13±0.04 ^{bB}	1.54±0.06 ^{bA}
	150	51.5±1.31 ^{aA}	35.8±1.67 ^{aB}	1.58±0.24 ^{aC}	1.87±0.06 ^{aA}
1 mM	Control	25.5±0.82 ^{bB}	20.2±1.36 ^{cA}	1.07±0.05 ^{cA}	1.30±0.07 ^{bA}
	50	40.6±4.22 ^{aA}	31.8±3.30 ^{bA}	1.60±0.09 ^{bA}	1.61±0.10 ^{abA}
	150	45.2±2.73 ^{aB}	50.2±0.75 ^{aA}	2.16±0.02 ^{aA}	1.94±0.13 ^{aA}
4 mM	Control	30.5±1.67 ^{cA}	22.5±2.40 ^{bA}	0.70±0.11 ^{cB}	1.33±0.10 ^{bA}
	50	37.2±2.61 ^{bA}	28.3±3.05 ^{abA}	1.06±0.21 ^{bB}	1.66±0.14 ^{cA}
	150	48.6±2.25 ^{aAB}	33.8±4.60 ^{aB}	1.25±0.11 ^{aB}	2.02±0.11 ^{aA}

In each column, significant differences among the three NaCl levels within each Si treatment are indicated by different lower case letters and those between different Si levels within each NaCl treatment are indicated by different uppercase letters (P<0.05).

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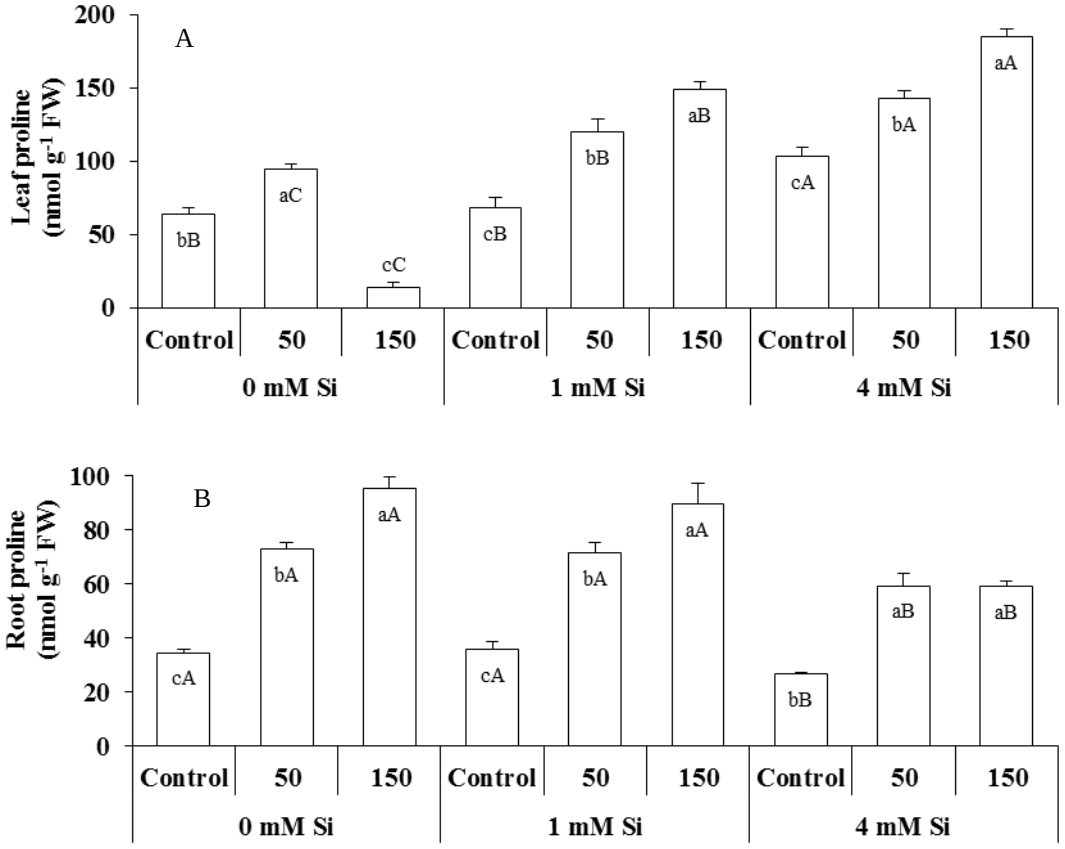


Figure 3. Concentrations of proline in the leaves (A) and roots (B) of wheat (*Triticum aestivum* L. cv. Homa) plants grown hydroponically under the three levels of NaCl salinity (control, 50, and 150 mM) and Si (as Na_2SiO_3) for two weeks. Significant differences among the three NaCl levels within each Si treatment are indicated by different lower case letters and those between different Si levels within each NaCl treatment are indicated by different uppercase letters (P<0.05).

alleviation of salt stress in such Si-accumulators as rice (Yeo *et al.*, 1999; Gong *et al.*, 2006), barley (Liang 1999; Liang *et al.*, 2007), zucchini (Savvas *et al.*, 2009), and cucumber (Zhu *et al.*, 2004) as well as such non-accumulators as tomato (Al-aghabary *et al.*, 2004; Romero-Aranda *et al.*, 2006), canola (Hashemi *et al.*, 2010), and tobacco (Hajiboland and Cheraghvareh, 2014). Authors have reported on the ameliorative effects of Si in wheat(Tahir *et al.*, 2006, Saqib *et al.*, 2008, Tuna *et al.*, 2008, Ali *et al.*, 2012, Ahmed *et al.*, 2013). This contrastive result is probably because of the short Si exposure time in our work. In the studies reported, the effects of Si were mainly observed during the whole growing season under field conditions and the reproductive organs were the most responsive parts to Si addition (Tahir *et al.*, 2006; Ali *et al.*, 2012; Ahmed *et al.*, 2013). It has been stated that the Si-induced physical barrier in roots is an important mechanism by which it mediates salt tolerance in accumulator plants (Zhu and Gong, 2014). It is likely that such Si deposits did not efficiently form in our experiment so that Si failed to affect the main components of salt-tolerance, particularly those related to the restriction of Na^+ entry to the roots and its shoot-root translocation (See below).

Effects of salt and Si on leaf pigment concentrations*:* Reductions in leaf Chl and Car induced by salt stress escalated upon Si addition. The higher leaf concentrations of Chl *a* observed in this work might have led to significant improvements in the net photosynthesis rate, as also previously reported for tobacco (Hajiboland and Cherghvareh, 2014). In addition, the enhanced leaf Car upon Si treatment might have played a great role in the improved salt tolerance in wheat. It has been argued that, under stress conditions such as salinity and drought, CO_2 limitation following stomatal closure inhibits photosynthetic biochemical reactions; producing an excessive excitation energy that causes both photoinhibition and damages to the photosystems. Heat dissipation from the excess excitation energy mediated by Car will, then, create the proper mechanism for quenching excess photons and protecting leaves (Hajiboland, 2014).

Salt stress increased leaf anthocyanin concentrations by about 4 fold but Si treatment affected it slightly. Anthocyanins are induced by a number of environmental factors including higher radiation, cold temperatures, water and osmotic stresses (Chalker Scott, 1999). Experiments in whole-plant systems have shown that anthocyanins accumulation is induced by saline conditions (Chalker-Scott, 1999; Hajiboland and Cherghvareh, 2014). Anthocyanins in leaf tissues have a dual function as absorbers of harmful levels and/or

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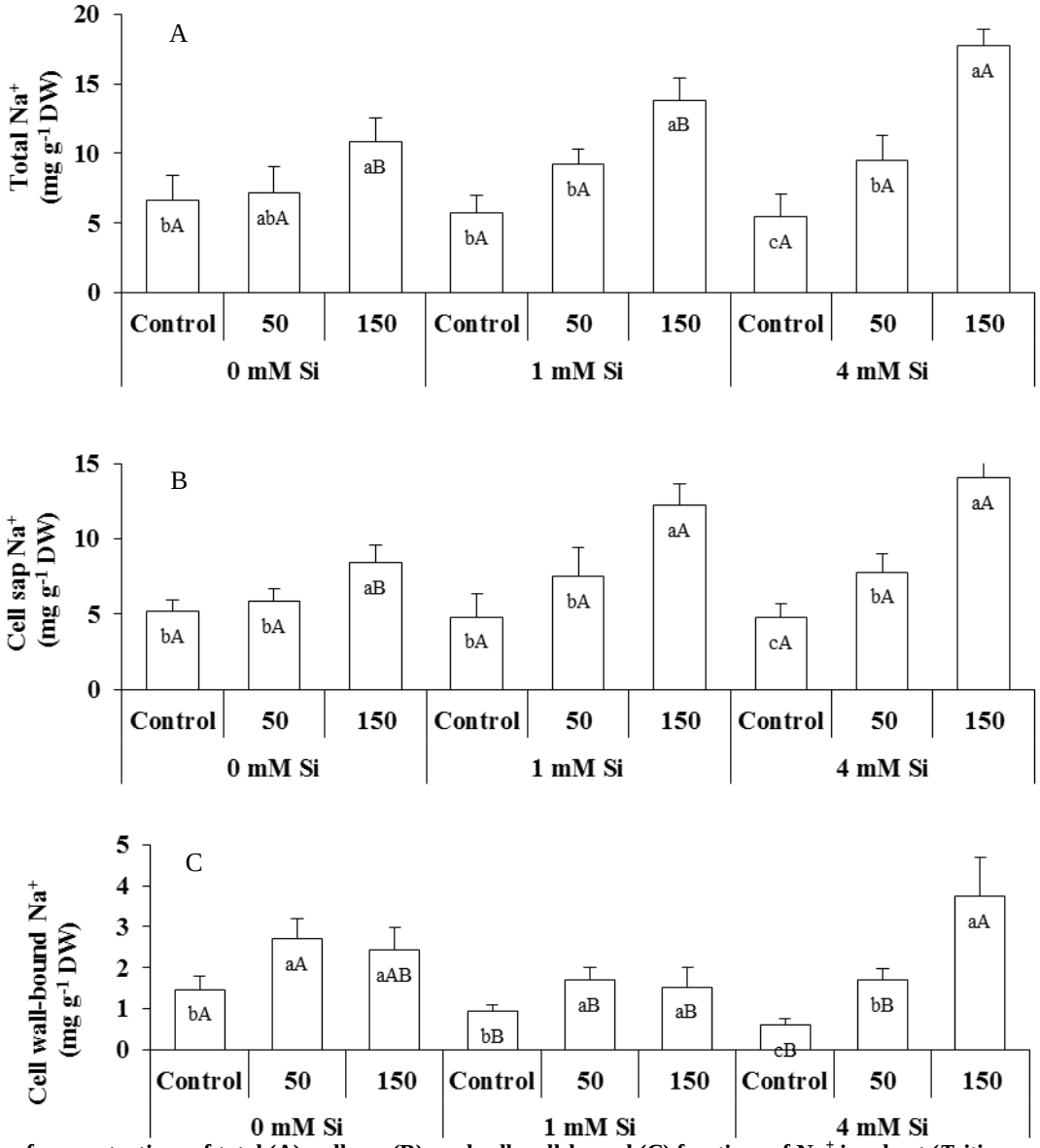


Figure 4. Leaf concentrations of total (A), cell sap (B), and cell wall-bound (C) fractions of Na^+ in wheat (*Triticum aestivum* L. cv. Homa) plants grown hydroponically under three levels of NaCl salinity (control, 50, and 150 mM) and Si (as Na_2SiO_3) for two weeks. Significant differences among the three NaCl levels within each Si treatment are indicated by different lower case letters and those between different Si levels within each NaCl treatment are indicated by different uppercase letters (P<0.05).

wavelengths of radiation and as osmotic adjusters (Chalker-Scott, 1999).

Effects of salt and Si on the accumulation of organic osmolytes: In addition to antioxidant accumulation, plants under stress nearly always accumulate compatible solutes, mainly including proline, glycine betaine, carbohydrates, and polyols (Rhodes *et al.*, 2002; Chen and Jiang, 2010). In the wheat plants tested in the present work, soluble carbohydrates increased in concentration in response to salt treatment in both leaves and roots while free amino acids reduced under the same conditions as did soluble proteins. This implies the different contributions by carbonated and nitrogenous low molecular weight osmolytes to the adaptation of wheat to salt stress. Si treatment was observed to lead to the accumulation of both compatible solutes in the leaves. Little is known, however, about the mechanism underlying Si-mediated changes in the metabolism of compatible solutes. Simultaneous accumulation of soluble sugars and starch in the leaves under salt stress probably stemmed from the plant growth which experienced a greater decline than did the photosynthesis and/or the depression of sink demand in the salt-stressed plants. In the Si-treated plants, however, a higher photosynthesis rate occurred probably due to the simultaneous enhancements in soluble sugars and starch concentrations with no reductions in plant growth.

Leaf concentration of proline exhibited a 13-fold increase upon Si treatment under severe salinity stress; unlike the other organic solutes examined, the high Si level in this experiment was indeed the optimum one for

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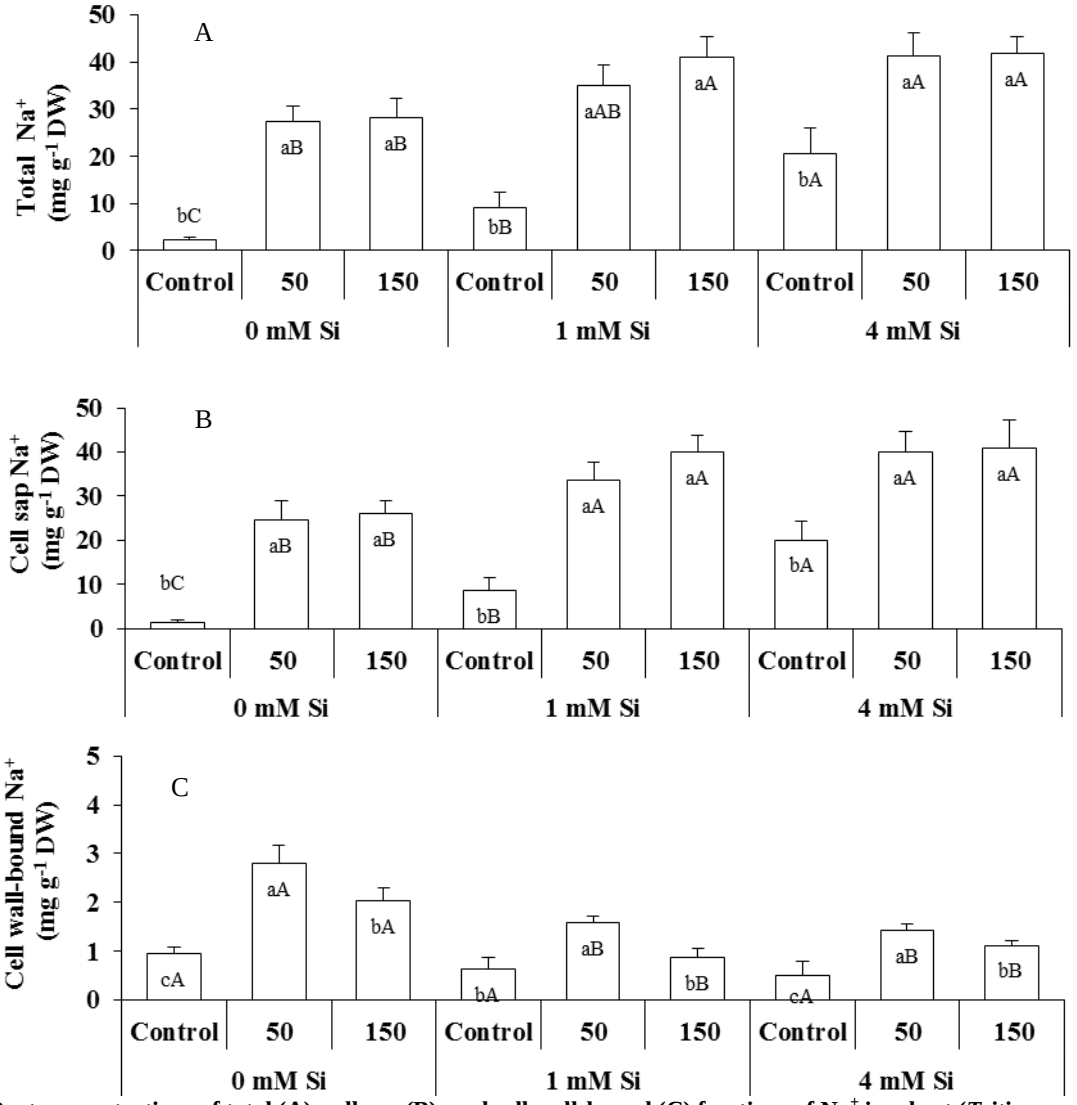


Figure 5. Root concentrations of total (A), cell sap (B), and cell wall-bound (C) fractions of Na^+ in wheat (*Triticum aestivum* L. cv. Homa) plants grown hydroponically under three levels of NaCl salinity (control, 50, and 150 mM) and Si (as Na_2SiO_3) for two weeks. Significant differences among the three NaCl levels within each Si treatment are indicated by different lower case letters and those between different Si levels within each NaCl treatment are indicated by different uppercase letters (P<0.05).

this effect. Proline, a nontoxic and protective osmolyte produced under osmotic stress, is frequently involved in osmotic protection and is reportedly associated with salt tolerance (Szabados and Savoure, 2009). In contrast to our findings, a number of studies have reported lowered proline levels as a result of Si addition in different salt-stressed plant species such as soybean (Lee *et al.*, 2010), wheat (Tuna *et al.*, 2008), barley (Gunes *et al.*, 2007), and sorghum (Yin *et al.*, 2013). Contradictory evidence has been reported on the accumulation of proline under salt and drought stress and effects of alleviating factors while both reductions and increases have been observed in proline concentration depending on the stress type and plant species (Ashraf and Harris, 2004).

In the roots, a significant accumulation of proline was observed upon salt treatment but it either remained unchanged as a result of Si (1 mM) addition or decreased (at 4 mM). Soluble sugar concentration, in contrast, was higher in the roots of Si-treated plants. This effect was not accompanied by a parallel increase in starch concentration, implying a limitation in carbohydrate accumulation in wheat roots under salt stress. The higher soluble proteins and amino acids observed in the Si-treated plants might have been the result of higher nitrate assimilation upon Si treatment; this is confirmed by similar observation reported on tobacco in both absence and presence of salt (Hajiboland and Cherghvareh, unpublished results). It is noteworthy that while proline accumulation was observed in the roots of salt-stressed wheat plants in this work, total fraction of amino acids did not accumulate in the roots, implying a specific effect of salt on proline biosynthesis (Ashraf and Harris, 2004; Ashraf and Foolad, 2007; Szabados and Savoure, 2009).

Effects of salt and Si on leaf and root osmotic potential and Na^+ concentration*:* Reduced growth

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under salt stress is the consequence of the high osmotic potential and Na^+ concentrations to toxic levels (Hasegawa *et al.,* 2000). In the wheat plants investigated in the present work, both leaf and root osmotic potentials decreased expectedly with both levels of salinity. Si treatment, however, did not increase leaf osmotic potential in the salt-treated plants, indicating that Si did not improve leaf water relation parameters under salt stress. In the roots, however, Si at 4 mM led to a significant increase in root osmotic potentials under a high salt concentration, which might have contributed to the enhance plant water uptake capacity under these conditions.

Si application may reportedly decrease Na^+ accumulation in the roots of barley (Liang, 1999) and alfalfa (Wang and Han, 2007), or in the leaves of rice (Gong *et al.,* 2006), sugarcane (Ashraf *et al.,* 2010), and sorghum (Yin *et al.,* 2013) as well as in both roots and leaves of wheat (Tuna *et al.,* 2008). This effect is likely to be mediated by an Si-induced increase in H^+ATPase and/or Na^+/H^+ antiporter activities under salt stress (Liang *et al.,* 2007; Zhu and Gong, 2014). The Si-induced physical barrier in the roots is another mechanism by which Si mediates salt tolerance in plants. In contrast to these reports, Na accumulation in wheat leaves and roots observed in the present study did not reduce with Si treatment; rather, the treatment increased Na^+ concentration under high salt concentrations. Dry weigh data indicated that Na^+ accumulation in the leaves and roots was not a concentration effect but that Si treatment increased both uptake and shoot-root transport of Na^+ under salt treatments. Si-mediated salt tolerance in plants is not always accompanied by a decrease in Na^+ levels in tissues. In tomato (Romero-Aranda *et al.,* 2006) and tobacco (Hajiboland and Cherghvareh, 2014), inclusion of Si reportedly had no significant effects on Na^+ concentration in leaves or roots. In rice, Si was observed to be deposited on the exodermis and endodermis of roots, which dramatically decreased apoplastic transport and transpirational bypass flow and, thereby Na^+ accumulation (Gong *et al.,* 2006). Our data, as was also confirmed by the findings of Gong *et al.* (2011), suggested that this mechanism may not work in other species. The greater Na^+ accumulation in the Si-treated wheat plants in the absence of salt might have been derived from the Na^+ present in the Si compound used in this work.

Na^+ distribution between the cell wall and cell sap fractions was found to be influenced by Si depending not only on salt and Si levels but also on the plant organ. In the leaves of plants under high salinity, Na^+ binding to the cell wall increased when the plants were supplemented with 4 mM Si. However, no such effect was observed under mild salinity or at lower Si levels. In the roots, a lower Na^+ binding to the cell wall was observed under both salinity and Si supplementation. Si alters cell wall properties and increases its capacity for binding cations such as Mn (Rogalla and Römheld, 2002) and Al (Wang *et al.,* 2004). An increase in Na^+ cell wall binding in response to 1 mM Si has been observed in the leaves of wheat plants grown hydroponically at 125 mM NaCl (Saqib *et al.,* 2008). The lack of this effect in the leaves under lower Si levels was likely because of the short Si exposure time in this work. To the best of the authors' knowledge, no published report is available on the effect of Na^+ binding to the cell wall in roots; this warrants further investigations to determine the contribution by this mechanism to Si-mediated alleviation of salt stress in plants.

Effects of Si on plant growth in the absence of salt stress*:* In the absence of salt, plant dry biomass was found to be significantly higher in plants supplemented with 1 mM Si. At a high Si level of 4 mM, the shoot did not continue its growth while the root did. Study has shown that the Si accumulator species grow better in the presence of Si (Epstein, 1994; Broadley *et al.,* 2012; Hajiboland, 2012). Such beneficial elements as Se, Al, Na, and Si owe their benefits to the mitigation of latent stress factors, activation of antioxidative defense, improvement of nutrients uptake, and water relation parameters (Pilon-Smits, *et al.,* 2009; Broadley *et al.,* 2012; Hajiboland, 2012). Si-mediated elevation of photosynthesis has been reported in both accumulator (Adatia and Besford, 1986) and non-accumulator (Hajiboland and Cheraghvareh, 2014) dicotyledonous species. In Gramineae, an additional mechanism may directly influence plant growth. Application of Si to rice, oats, wheat (Hossain *et al.,* 2002), and sorghum (Hattori *et al.,* 2003) was reported to lead to higher leaf (Hossain *et al.,* 2002) and root (Hattori *et al.,* 2003) growths compared to the control plants; this effect was attributed to an increase in cell wall extensibility in the apical regions. The present data indicate that the greater growth in Si-treated wheat plants in the absence of salt could be attributed to the improved leaf water status, the greater root water uptake capacity, and the higher leaf Chl, Car, and proline contents. Upregulation of carbon and nitrogen assimilation as reflected in the greater amounts of carbohydrates and nitrogenous compounds could be regarded as another mechanism stimulating Si-mediated growth in wheat.

Conclusion:

Salt stress-induced depression of plants biomass was higher in the +Si plants than in the –Si ones due to the higher biomass of the respective control (+Si) plants. The majority of the biochemical parameters investigated showed similar changes at both salinity levels. Leaf proline concentration, however, increased under mild salinity but decreased under high salinity, indicating a limitation for proline biosynthesis and its reduced contribution to the protection of cells under high salt concentrations in wheat. This limitation, however, was not observed in the +Si plants.

The beneficial effects of Si application on the improvement of plant biomass as well as increasing Car

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and leaf proline concentrations were not different between the two levels of Si applied. However, 1 mM Si reduced the osmotic potential of the leaves but increased leaf Chl *a*, protein, sugars, and starch contents; high Si level either had no such effects or led to reduced values of these parameters. Overall, the data obtained in this study suggest that Si at 1 mM is the optimum concentration for wheat plants under both stressed and non-stressed conditions.

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