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Full Length Research Paper

The alleviation of salinity induced stress with applications of silicon in soilless grown *Lactuca sativa* L. ‘Eish!’

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The effects of 30 and 60 mM NaCl on Lettuce (*Lactuca sativa* L. ‘Eish!’), grown in soilless culture, with additions of 0, 1, 2 and 4 mM Si was evaluated. Height, leaf number, weight, chlorophyll content and elemental analysis of plants were examined. Fresh root and shoot weight significantly increased with additions of 2 mM Si compared to that of the 60 mM NaCl control, with notable increases with Si treatments of 1 and 4 mM. Dry root and shoot weight with additions of 1 mM Si, and in shoot weight with additions of 2 mM Si, significantly increased when compared to the 60 mM NaCl control. Increases in dry root and shoot weight of the other Si additions lead to clear, yet insignificant increases in dry weights. Few differences in Cl content were evident amongst treatments, besides a significant increase in root content and a significant decrease in shoot content with applications of 4 mM Si exposed to 60 mM NaCl. Na shoot content of 1, 2 and 4 mM Si at 60 mM NaCl showed a significant decrease compared to that of the control. Applications of 2 and 4 mM Si showed increased shoot Si when compared to both the 30 and 60 mM NaCl controls. With clear evidence for a reduction in Na content in shoots with applications of Si at higher NaCl concentrations, and further increases in plant weights, it can be stated that applications of Si are of a distinct benefit for the growth of lettuce.

Key words: Lettuce, Si, NaCl, salt, sodium chloride, hydroponics.

INTRODUCTION

Silicon [Si], an element whose abundance in the earth’s crust is second only to oxygen (Marshak, 2005), can contribute as much as 0.1 to 10% of the dry matter of plants. These levels are equal to or exceeding those of essential macro nutrients (Epstein and Bloom, 2005). Si has in the past been classified as a non-essential element for plant nutrition, that is, one which does not meet the criteria for essentiality in the classic definition of essentiality proposed by Arnon and Stout (1939). More recently Si has been classified as either a beneficial element (Marschner, 1995), or as a quasi-essential element in plants (Epstein, 1999).

Contributing to its classification as a beneficial or quasi-essential element in plants is its role as a plant stress ameliorator (Epstein, 1994, 1999; Belanger et al., 1995). An important aspect of this is the ability of Si to ameliorate salt toxicity, defined as conditions of high salt build-up, with sodium chloride [NaCl] being the most common cause of saline conditions (Harris, 1992; Epstein and Bloom, 2005).

Salinity has two methods of causing abnormalities in plant growth. The first, which is independent of the type of salt, is by creating a higher osmotic potential in the medium surrounding the roots, and because osmosis is the principle method of water absorption in plants (Stern, 2006), it results in a decrease in plant-water availability (Carter, 1981). The second, which is salt dependent, is

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caused by an excess of available ions. In the case of NaCl salinity, the ions in question are sodium [Na^+] and chloride [Cl^-]. Cl is known to be an essential micronutrient in plants (Epstein and Bloom, 2005; Stern, 2006), and some argued that Cl should be classified as a macronutrient, contributing as much as 1% of the dry weight of some plants (Muckle, 1993). Na, on the other hand, is required by only a few plants (Epstein and Bloom, 2005), and has been shown to be the primary cause of growth suppression in lettuce (Tas et al., 2005). In general, high concentrations of both Na and Cl are associated with leaf scorching (Shannon and Grieve, 1999), necrosis and defoliation (Maas and Hoffman, 1977).

The mitigation of NaCl induced toxicity with the application of Si has been demonstrated in many important agricultural and ornamental crops, including Alfalfa (Wang et al., 2011), tomato (Romero-Aranda et al., 2006), wheat (Ahmad et al., 1992), zucchini (Savvas et al., 2009), grapevine (Soylemezoglu et al., 2009), mesquite (Bradbury and Ahmad, 1990), roses (Savvas et al., 2007; Reezi et al., 2009) and several others. The present study investigated the ability of Si to alleviate NaCl toxicity in lettuce (*Lactuca sativa* L. 'Eish!').

MATERIALS AND METHODS

The experiment took place within the experimental greenhouse located at the Cape Peninsula University of Technology in Cape Town, South Africa - 33° 55' 58.27''S, 18° 25' 57.04 E. The temperature within the greenhouse ranged from 9 to 29°C, the mean being 17.6°C and the humidity ranged from 44 to 99%, the mean being 77%.

L. sativa L. 'Eish!' seeds (Hygrotech, South Africa) were sown in vermiculite, and germinated under 40% shade-cloth. Once all seeds had germinated (8 days after sowing) they were fertilised daily with half strength CHEMICULT (Starke Ayres, South Africa) nutrient solution, and increased gradually to full strength (Harris, 1992). Once the seedlings were fully established (40 days after sowing) 80 plants were randomly selected and transplanted into individual 12.5 cm pots, containing expanded clay as the medium, and placed within the soilless growing system. The sub-irrigation, closed, soilless growing system consisted of a nutrient solution holding tray, delivering a constant ± 1 cm of circulating nutrient solution to each treatment. Using a randomised block design, 10 plants were placed within eight individual soilless culture units, allowing for 10 repeats of each.

Basal nutrient solution

Analytical grade, potassium nitrate [KNO_3], calcium nitrate [$(Ca(NO_3)_2 \cdot 4H_2O$], ammonium dihydrogen phosphate [$NH_4H_2PO_4$], and magnesium sulphate [$MgSO_4 \cdot 7H_2O$], were used to add 16 mM N, 6 mM K, 4 mM Ca, 2 mM P, 1 mM S and 1 mM Mg to the nutrient solution, which was based on the modified Hoagland solution presented by Epstein and Bloom (2005). Micro nutrients were supplied by using HYGROPLEX (Hygrotech, South Africa), adding 58 µM Boron [B], 11.4 µM manganese [Mn], 7.6 µM zinc [Zn], 1 µM copper [Cu], 0.65 µM molybdenum [Mo] and 31.9 µM iron [Fe] to the nutrient solution. The complete basal nutrient solution was prepared using deionised water and had an electrical conductivity (EC) of 1.9 mS cm^{-1}.

Silicon and NaCl treatments

Four levels of Si, 0, 1, 2 and 4 mM, were supplied by adding AGRISIL K50 (PQ Silicas, South Africa) - Potassium silicate [K_2SiO_3], each of which were combined with two levels of NaCl, 30 and 60 mM, making a total of 8 treatments. Treatments, which were applied in a single dose, commenced one week after transplantation. Newly prepared nutrient solutions containing 30 mM NaCl had a mean EC of 5.20 mS cm^{-1} and solutions containing 60 mM NaCl had a mean EC of 8.10 mS cm^{-1}. These EC levels were maintained daily. Potassium [K] was balanced in all treatments by subtracting additional K from KNO_3, and supplementing lost Nitrate [NO_3] by adding nitric acid [HNO_3]. The nutrient solution, which was replaced weekly, was maintained daily at a pH of 6.0 using hydrochloric acid [HCl] and sodium hydroxide [NaOH].

Plant growth parameters

Plant growth, in terms of plant height and leaf number, of all treatments, were measured at weekly intervals. Plant height was measured with a measuring tape, from the surface of the medium to the tip of the tallest leaf.

Five plants per treatment were harvested after 11 weeks from sowing (Hadfield, 2001), and separated into roots and shoots. Roots were rinsed once with tap water and twice with deionised water before being patted dry with paper towel. After the fresh weight had been recorded, plants were immediately placed within a specimen oven at 60°C until constant weight, and the dry weight recorded.

Determination of chlorophyll content in plant leaves

Extraction of chlorophyll by dimethyl sulphoxide [DMSO] was done following a modified method described by Hiscox and Israelstam (1979). This method allows for the extraction of chlorophyll without plant tissue maceration. Five lettuce plants per treatment were lyophilised and ground to a fine powder using a mortar and pestle and stored at -80°C until analysed. 100 mg of the powder was placed in a 15 ml vial containing 7 ml DMSO and incubated at 65°C for 20 min. After the incubation period, an additional 3 ml of DMSO was added to the vial, and stored at 4°C. After a 24 h storage period, the extract was centrifuged at 3220 g for 10 min, after which the supernatant was transferred to a new 15 ml vial. Before spectrophotometrical analysis, the supernatant was diluted to a factor of 15 of which 1 ml was placed in a cuvette and absorbance values of 645 and 663 nm recorded. Absorbance values were used in the equation proposed by Arnon (1949), to determine total leaf chlorophyll content against DMSO blank, expressed as mg g^{-1} dry weight.

Elemental analysis

Dried plant material was used to analyse for Na, Cl and Si. Na and Si was analysed by ashing the ground sample and dissolving the ash in HCl. Elemental concentrations were determined using an inductively-coupled plasma (ICP) emission spectrophotometer. Cl was analysed by digesting the dried, ground sample with HNO_3. An excessive quantity of silver nitrate [AgNO3] was added, followed by back titration with potassium thiocyanate [KSCN]. Elemental concentrations of Na, Cl and Si are expressed as mg g^{-1} of dried plant material (A. Van Deventer, Bemlab, South Africa, Personal Communication).

Statistical analysis

Statistical analysis was performed by using two-way independent

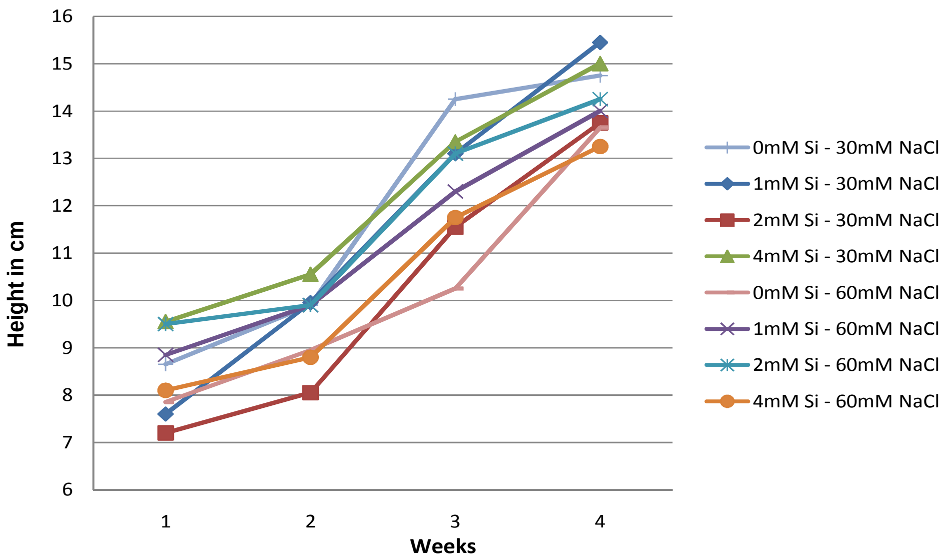

Figure 1. The effect of 30 and 60 mM NaCl on plant height, with additions of 0, 1, 2 and 4 mM Si. Measurements were taken weekly from 27 May (1) to 17 June (4) 2011.

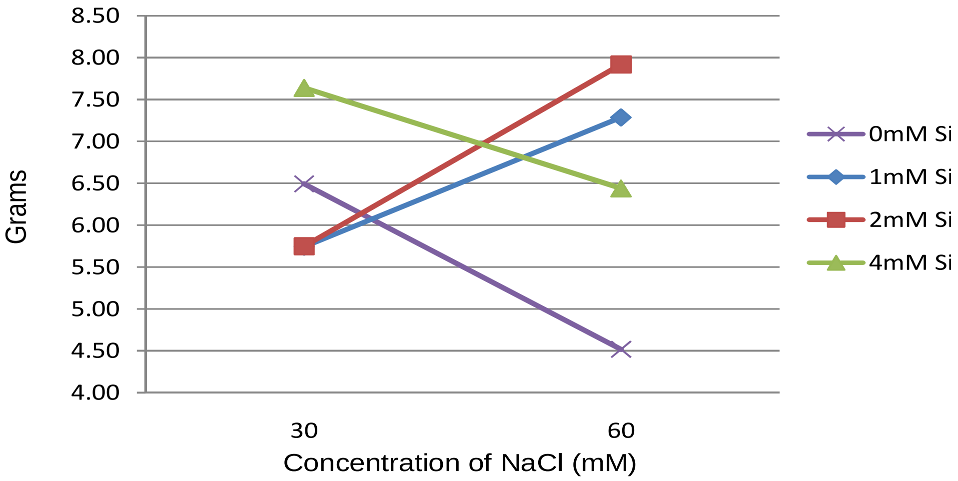

Figure 2. The effect of 30 and 60 mM NaCl on fresh root weight, with additions of 0, 1, 2 and 4 mM Si.

analysis of variance (ANOVA) followed by the Bonferroni post test, with Si and NaCl concentrations being the factors assessed. Computations were executed with the software program SPSS (Urdan, 2005).

RESULTS

Leaf number showed no significant differences between Si treatments, whether compared to the control, containing 0 mM Si, or to each other (data not shown). Plant height showed similar insignificance between treatments and controls, besides the expected general increase in height over the growing period (Figure 1). There was a significant interaction between salt concentration and silicon treatments on fresh root weight ($P \leq 0.05$) and fresh shoot weight ($P \leq 0.1$). Main effect analysis showed a significant decrease in fresh root weight and fresh shoot weight ($P \leq 0.1$ and $P \leq 0.05$ respectively) between the two controls (Figures 2 and 3). Fresh root weight and fresh shoot weight showed no significant differences at Si levels of 1 and 4 mM whether grown with 30 or 60 mM NaCl, when compared to their respective control. The treatment of 2 mM Si exposed to 60 mM did however show a significant increase in fresh root ($P \leq 0.05$) and shoot weight ($P \leq 0.1$) when compared to the control. Although only 2 mM Si showed a significant effect on

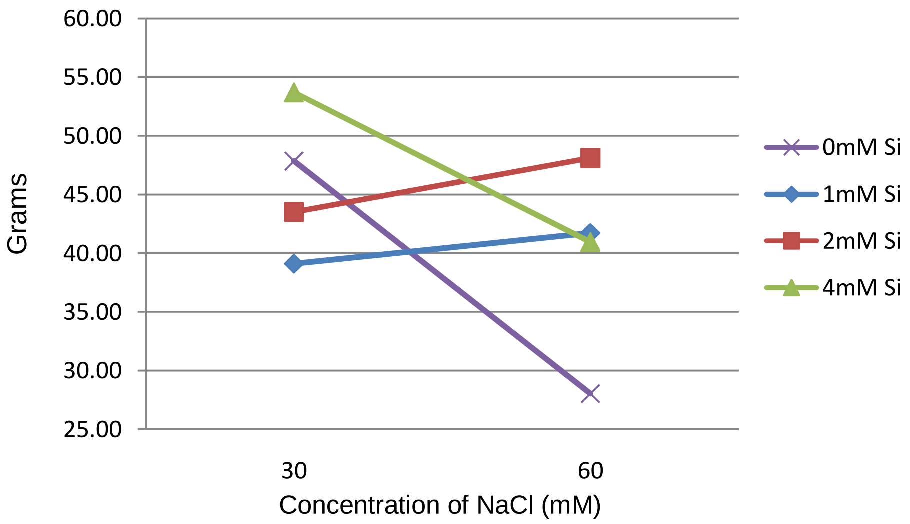

Figure 3. The effect of 30 and 60 mM NaCl on fresh shoot weight, with additions of 0, 1, 2 and 4 mM Si.

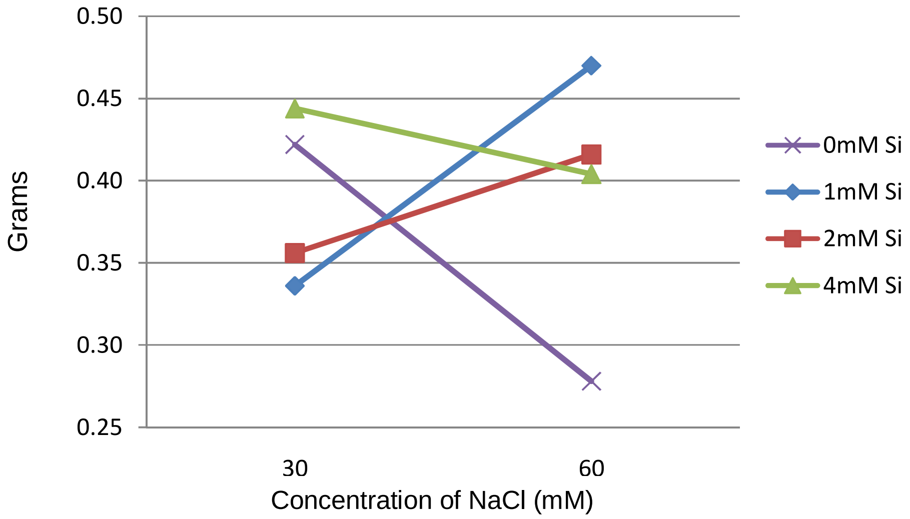

Figure 4. The effect of 30 and 60 mM NaCl on dry root weight, with additions of 0, 1, 2 and 4 mM Si.

fresh root and shoot weight, there was a marked, yet insignificant, increase in fresh root and shoot weight of 4 mM Si at 30 mM NaCl, and 1 mM and 4 mM Si at 60 mM when compared to the control (Figures 2 and 3).

There was also a significant interaction between salt concentration and silicon treatments on dry root weight ($P \leq 0.1$) and dry shoot weight ($P \leq 0.05$). Main effect analysis showed that dry root weight significantly decreased ($P \leq 0.1$) between the control of 30 mM NaCl and that of 60 mM NaCl. Besides a significant increase ($P \leq 0.1$) in dry root weight between 1 mM Si exposed to 60 mM NaCl and that of the control, the other treatments showed no significant differences, even though there was again a marked increase in weight, especially when compared to the 60 mM NaCl control (Figure 4). Dry shoot weight showed no significant increase between controls. However, there were significant increases ($P \leq 0.05$) in weight of 1 and 2 mM Si when compared to the60 mM NaCl control. Again, although other treatments resulted in insignificant differences, there was a marked increase in root and shoot dry weight when compared to that of the control (Figure 5).

Although there was a significant interaction between salt concentration and silicon treatments on root ($P \leq 0.001$)

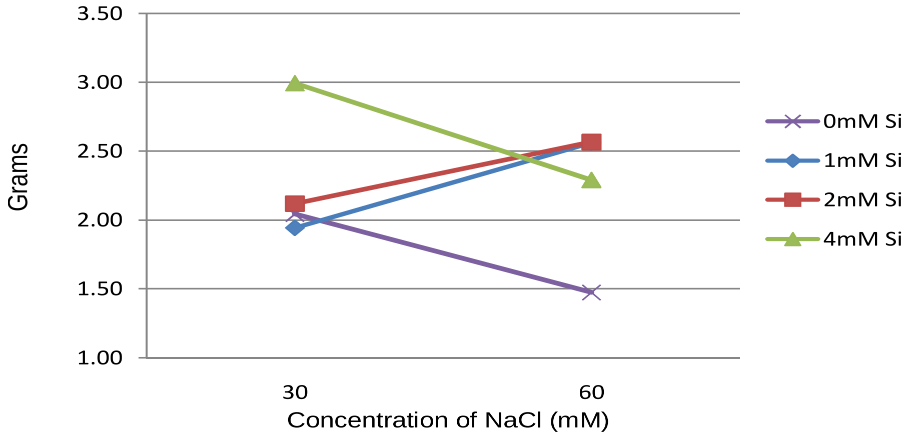

Figure 5. The effect of 30 and 60 mM NaCl on dry shoot weight, with additions of 0, 1, 2 and 4 mM Si.

Table 1. The effect of 30 and 60mM NaCl on Cl, Na and Si root and shoot content, with additions of 0, 1, 2 and 4mM Si.

Treatment		**Cl (mg g^{-1})**		**Na (mg g^{-1})**		**Si (mg g^{-1})**	
NaCl (mM)	**Si (mM)**	**Root**	**Shoot**	**Root**	**Shoot**	**Root**	**Shoot**
30	0	12.18	16.78	7.17	11.11	0.42	0.06
	1	9.78	18.70	8.12	11.41	0.44	0.12
	2	9.60	22.54	8.59	11.22	0.51	0.15**
	4	14.42	13.74	8.10	11.64	0.43	0.43***
60	0	15.98	21.20	14.30	16.88	0.37	0.07
	1	14.82	13.62	9.43	13.75***	0.29	0.11
	2	7.66	14.20	10.77	15.40**	0.45	0.16***
	4	74.8***	12.74*	8.83	14.00***	0.40	0.18***

and shoot ($P \leq 0.05$) Cl content, main effect analysis showed that Cl concentrations only increased significantly in roots of the 4 mM Si with 60 mM NaCl treated plants and the shoots of 2 mM Si plants. This in turn corresponded to a significant decrease ($P \leq 0.1$) in shoot Cl when compared to the control at 60 mM NaCl (Table 1). When comparing Cl content of plants treated with the same level of Si, 2 mM Si at 60 mM NaCl showed a significant decrease in shoot Cl content ($P \leq 0.05$) compared to the plants treated with 30 mM NaCl. Contrarily, plants treated with 4 at 60 mM NaCl showed a significant increase in root Cl content ($P \leq 0.01$) when compared to the plants treated with 30 mM NaCl (Table 2).

There was a significant interaction between salt concentration and silicon treatments on shoot Na content ($P \leq 0.001$) but not on root Na content. Although no significant differences in root Na content were obtained, there was a marked decrease in Na root concentration at 60 mM NaCl (Figure 6). Main effect analysis showed that Na shoot content at 30 mM NaCl mirrored the results of that of the roots, and showed no significant differences between treatments. However, Na shoot content at 60 mM NaCl with Si levels of 1, 2 and 4m M all resulted in a significant decrease in shoot Na content compared to that of the control (Table 1). When comparing Na content of plants treated with the same level of Si, root Na significantly increase between the controls of 30 and 60 mM NaCl ($P \leq 0.001$) and shoot Na significantly increase ($P \leq 0.001$) with 60 mM NaCl when compared to 30 mM NaCl at all Si levels (Table 2).

There was a significant interaction between salt concentration and silicon treatments on Si shoot content ($P \leq 0.001$), but not on Si root content. Main effect analysis showed that there was however a significant decrease ($P \leq 0.05$) of root Si between treatments at 30 and 60 mM NaCl receiving 1 mM Si. Si shoot concentrations of 2 and 4 mM, at both 30 and 60 mM NaCl showed a significant increase when compared to the control. When the two NaCl levels at the same 4 mM Si treatment were compared, a significant decrease ($P \leq 0.01$)

Table 2. The effect of 30 and 60 mM NaCl on Cl, Na and Si root and shoot content, with additions of 0, 1, 2 and 4 mM Si.

Treatment		Cl (mg g^{-1})		Na (mg g^{-1})		Si (mg g^{-1})	
Si (mM)	**NaCl (mM)**	**Roots**	**Shoots**	**Roots**	**Shoots**	**Roots**	**Shoots**
0	30	12.18	16.78	7.17	11.11	0.42	0.06
	60	15.98	21.20	14.30***	16.88***	0.37	0.07
1	30	9.78	18.70	8.12	11.41	0.44	0.12
	60	14.82	13.62	9.43	13.75***	0.29**	0.11
2	30	9.60	22.54	8.59	11.22	0.51	0.15
	60	7.66	14.20**	10.77	15.40***	0.45	0.16
4	30	14.42	13.74	8.10	11.64	0.43	0.43
	60	74.8***	12.74	8.83	14.00***	0.40	0.18***

Values presented are means ± SE, n = 5. *; **; *** = significant at $P \leq 0.1$*, $P \leq 0.05$** or $P \leq 0.01$***. Significance compared to that of the same Si concentrations, with different NaCl concentrations.

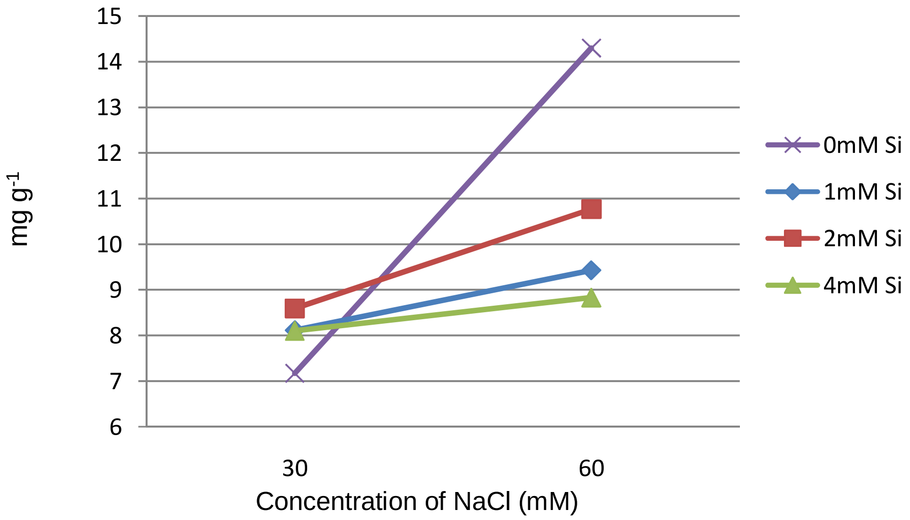

Figure 6. The effect of 30 and 60 mM NaCl on Na root content, with additions of 0, 1, 2 and 4 mM Si.

in Si was observed in the shoots. This was apparent in the 4 mM treatment alone (Table 2).

There was a significant interaction between salt concentration and silicon treatments on chlorophyll content ($P \leq 0.05$). Main effect analysis revealed that, when comparing Si treated plants to the controls, there was only a significant decrease in chlorophyll with applications of 2 mM Si at 30 mM to that of the control (Figure 7).

DISCUSSION

The results obtained from this study support the fundamental findings of previous studies, that Si has beneficial mitigating properties with regards to NaCl induced salinity stress. These findings indicate some benefits of Si applications at 30 mM NaCl; however, the benefits of Si seem to be more apparent with the higher experimental concentrations of 60 mM NaCl. This is evident when looking at the significant increase in fresh root and shoot weight at 2 mM Si at 60 mM NaCl, the significant increases in dry root, at 1 mM Si, dry shoot weight at 1 and 2 mM, and the overall marked increases in fresh and dry weights when comparing all Si treatments to that of the 60 mM NaCl control (Figures 2 to 5). These findings are supported by the literature which

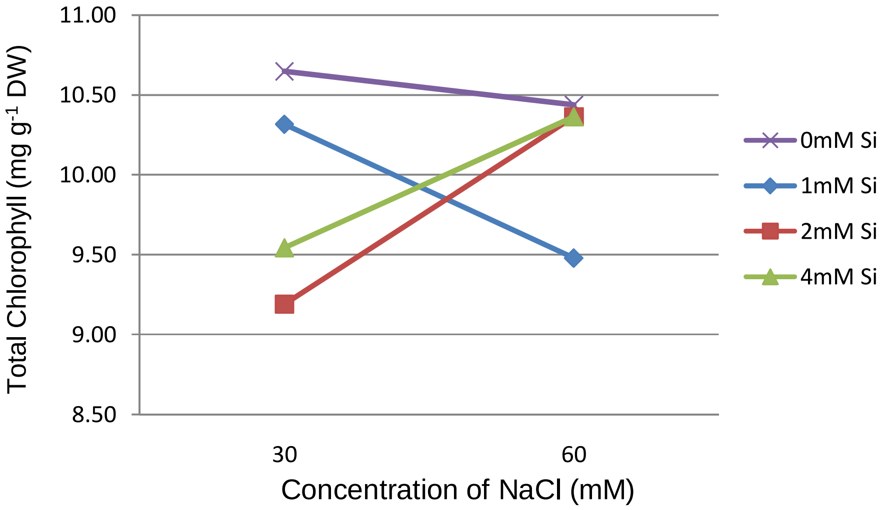

Figure 7. The effect of 30 and 60 mM NaCl on total chlorophyll content with additions of 0, 1, 2 and 4 mM Si.

states that dry shoot and root weights of plants increase with applications of Si compared to a range of NaCl concentrations lacking Si (Romero-Aranda et al., 2006; Tuna et al., 2008; Ashraf et al., 2010).

Chlorophyll content showed no significant differences, with only one exception with an application 2 mM Si at 30 mM NaCl (Figure 7). This is in line with Tas et al. (2005), who although did not investigate Si, reported negligible effects of NaCl on chlorophyll content on lettuce. However, in a study conducted by Hashemi et al. (2010), evaluating the mitigating effects of Si on Canola, a vast increase in chlorophyll content was found.

A notable oddity with regards to elemental analysis was the exceptional accumulation of Cl in the roots of the plants treated with 4 mM Si at 60 mM (Tables 1 and 2). This, when compared to the control, corresponded to a significant decrease in Cl accumulation in shoots. Savvas et al. (2007, 2009) found similar results, reporting a significant decrease in shoot Cl content with Si applications. However, Romero-Aranda et al. (2006), and Soylemezoglu et al. (2009), reported a slight increase in Cl shoots concentration with applications of Si.

A significant finding with regards Na content was the reduction of Na within shoots when Si treated plants was exposed to 60 mM NaCl when compared to the control (Table 1). The decrease of Na content in Si treated plants has been a major finding when evaluating the ability of Si to mitigate NaCl salinity stress. Many studies that evaluate Si and NaCl relate NaCl mitigation to a reduction in Na shoot concentrations (Saqib et al., 2008; Tuna et al., 2008; Savvas et al., 2009; Ashraf et al., 2010), the reduction of which may be linked to Si reducing transpiration, as concluded by Ma et al. (2001). Savvas (2007), also reported a decrease in Na with applications of Si, but did not conclude this to be factor influencing the ability of Si to mitigate salinity stress.

Other mechanisms of salinity mitigation by Si have been suggested, including the ability of Si to improve water storage within plant tissues. Due to the higher growth rate that this would allow, it follows that salt concentrations would decrease through dilution, and therefore ameliorate salt toxicity (Romero-Aranda et al., 2006). Anti oxidants have also been a topic of investigation, with Liang (1999) and Zhu et al. (2004a, b), investigating the interaction of Si with antioxidants in salt stressed barley, cucumber and tomato respectively, with promising results.

Although transpiration and anti oxidants were not factors investigated in this study, plant weight increases, and subsequent decreases in Na shoot concentrations, leads to the conclusion that mitigation of NaCl induced salinity with applications of Si was at least partially due to the reduction of shoot Na content. Additions of Si to plants under salinity stress can be recommended, especially at higher salinity levels, as shown by the results of this paper.

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