Optimizing silicon application to improve salinity tolerance in Maize

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Abstract

Salinity often suppresses the wheat performance. As wheat is designated as silicon (Si) accumulator, hence Si application may alleviate the salinity induced damages. With the objective to combat the salinity stress in wheat by Si application (0, 50, 100, 150 and 200 mg L^{-1}) using calcium silicate, an experiment was conducted on two contrasting wheat genotypes (salt sensitive; Auqab-2000 and salt tolerant; SARC-5) in salinized (10 dS m^{-1}) and non-salinized (2 dS m^{-1}) solutions. Plants were harvested 32 days after transplanting and evaluation was done on the basis of different morphological and analytical characters. Silicon supplementation into the solution culture improved wheat growth and K^+/Na^+ with reducedNa$^+$ and enhanced K^+ uptake. Concomitant improvement in shoot growth was observed; nonetheless the root growth remained unaffected by Si application. Better results were obtained with 150 and 200 mg L^{-1} of Si which were found almost equally effective. It was concluded that SARC-5 is better than Auqab-2000 against salt stress and Si inclusion into the solution medium is beneficialfor wheat and can improve the crop growth both under optimal and salt stressful conditions.

Key words: Wheat, salt tolerance, Si, solution culture, growth

Introduction

Plant response to salt stress conditions is a complex mechanism that is not fully understood. The response of plants to excessive salinity is multifaceted involving changes in plant's morphology, physiology and metabolism (Rhoades, 1993, Hilal *et al*., 1998), ultimately diminishing growth and yield (Ashraf and Harris, 2004). Excess of soluble salts in root zone negatively affects plant growth and yield through osmotic effects, nutritional imbalances and specific ion toxicities (Grattan and Grieve, 1999; Munns, 2005; Tahir *et al*., 2006). Wheat, a glycophytic plant, is adversely affected by salinity stress (Zhu, 2003). Yield losses up to 45% have been reported due to salinity stress in wheat (Qureshi and Barrett-Lennard, 1998).

Studies on salt tolerance often point to restricted ion accumulation (Greenway and Munns, 1980). Exogenous application of nutrients has been found to improve the crop performance under salt stress (Raza *et al*., 2006). The damaging effects of salts have been ameliorated with exogenous application of K^+ in wheat (Akram *et al*., 2007), N in *Phaseolus vulgaris* (Wagenet *et al.*, 1983) and Ca in snap bean (Awada *et al.*, 1995). Furthermore, some beneficial mineral nutrients have been studied that can counteract adverse effects of salt stress. Silicon (Si),being a beneficial element provides significant benefits to plants at various growth stages (Epstein, 1999).

Silicon accumulates in plants at a rate comparable to those of macronutrient elements like calcium, magnesium and phosphorous (Epstein, 1999). Wheat is also classified as Si accumulator. It is evident that Si is beneficial for the growth of many plants under various abiotic (e.g. salt, drought and metal toxicity) and biotic (plant diseases and pests) stresses (Liang *et al*., 2003; Ma, 2004). Some possible mechanisms through which Si may increase salinity tolerance in plants (Liang *et al*., 2003) include: increased plant water status (Romero *et al*., 2006),; stimulation of reactive oxygen species (ROS) (Zhu *et al*., 2004); immobilization of toxic Na^+ ion (Liang *et al*., 2003); reduced Na^+ uptake in plants and enhanced K^+ uptake (Yeo *et al*., 1999; Liang *et al*., 2005) and higher K^+: Na^+ selectivity (Hasegawa *et al*., 2000). Hence, Si has vital importance for better plant growth under salinity.(Tahir *et al*., 2006).

The purpose of the current study was to optimize, the level of Si for wheat crop under saline environment and present some experimental evidence about the role of Si in crop biology. To achieve this purpose, the effects of Si were assessed for two genotypes contrasting in salinity tolerance. The hypothesis was to verify whether Si may be useful to enhance the salt tolerance of wheat that is mediated via improvement in plant growth.

Materials and Methods

The experiment was conducted in rain protected net house in hydroponics following completely randomized design with three replicates in factorial arrangement. Average temperatures in the net house were 20±7ºC during the day and 12±5ºC at night time during the experimental period. The relative humidity remained between 50% (midday) to 85% (midnight). Light intensity ranged between 350 and 1400 µmol photon m^{-2} s^{-1} depending upon the day and cloud conditions.

Seeds of two wheat genotypes Auqab-2000 (salt sensitive) and SARC-5 (salt tolerant), used in this study were obtained from Ayub Agriculture Research Institute, Faisalabad, Pakistan and Saline Agriculture Research Centre, University of Agriculture, Faisalabad, Pakistan, respectively. Five different levels of Si (0, 50, 100, 150 and 200 mg L^{-1}) were used along with two salinity levels (2 dS m^{-1} and 10 dS m^{-1}).

Wheat seeds were grown in sand filled plastic pots (Diameter = 45cm). Two weeks after sowing, 15 uniform size seedlings were transferred to plastic tubs (Radius =14cm, Height =19cm, Volume=11.693 L) having continuously aerated half strength Johnson's nutrient solution (Johnson *et al.*, 1957) by fixing with thermopal sheets at the top. The required salinity (EC) was developed by adding NaCl (National refined salt with 99.10% purity) in distilled water and EC was measured with Mi-70 (Benchmeter EC/TDS/NaCl/Temperature). Calcium silicate was applied at the time of transplantation after dissolving it with KOH at 71°C on a hot plate. Hydrogen ion activity (pH) of the solution was monitored and adjusted daily at 6.5 to 5.5 and solution was changed weekly.

Determination of growth parameters

Plants were harvested 32 days after transplanting and separated into roots and shoots. Root and shoot lengths and fresh weights were measured immediately, while to record dry weights, the samples were oven-dried till constant weights.

Determination of Na^+ and K^+ from flag leaves

The oven dried and ground material (0.1 g) of leaves was digested with mixture of 2 mL of sulfuric acid and hydrogen peroxide according to the method of Wolf (1980). Potassium and sodium in the digested material were determined with a flame photometer (Jenway, PFP-7).

Digestion

The dried ground material (0.1 g) was taken in digestion tubes, 2 mL of conc. H_2SO_4 were added and were incubated overnight at room temperature. Then 1 mL of H_2O_2 (35% A. R. grade extra pure) was poured down through the sides of the digestion tubes. After waiting for the reaction, the tubes were ported in a digestion block and heated upto 350 ºC until fumes were produced and continued to heat for further 30 min. Digestion tubes were removed from the block and cooled. One mililiter of H_2O_2 was slowly added and the tubes were placed back into the digestion block until fumes were produced for 20 min. Digestion tubes were removed again and above mentioned step was repeated until the cooled material was colorless. The volume of extracts was made 50 mL with distilled water, filtered and used for the determination of mineral elements.

Determination of Si from flag leaf

The leaves of harvested plants were oven dried and ground in a Wiley mill built-in with stainless steel chamber into fine powder. The ground samples (0.5g) were digested in 2 mL 50 % hydrogen peroxide (H_2O_2) and 4.5 g 50 % NaOH in open vessels (Teflon beakers) on a hot plate at 150 ºC for 4 hours. Si concentration was measured using calorimetric amino molybdate blue color method (Elliot and Synder, 1991). To 1mL of supernatant filtrate liquid, 10 mL of ammonium moblybdate (54g L^{-1}) solution and 25 mL of 20 % acetic acid was added in 50 mL of polypropylene volumetric flask. After five minutes, 5 mL of 20 % tartaric acid and 1 mL of reducing solution was added in flask and volume was made with 20 % citric acid. After 30 minutes, the absorbance was measured at 650 nm wave length with a UV visible spectrophotometer (Shimdzu, Spectronic 100, Japan). The reducing agent was prepared by dissolving 0.5 g 1 amino-2-naphthol-4-sulfonic acid, 1 g Na_2SO_3 and 30 g $NaHSO_3$ in 200 mL water (Elliott and Synder, 1991)

Results

Mean squares in pooled ANOVA (Table-1) showed that the highest variation in growth of wheat occurred due to variation in salinity levels followed by Si and genotypes. Interactive effect of variety x Si was non-significant in all observed traits, Salinity x Variety caused greater change in growth followed by Salinity x Si and Salinity x Variety x Si.

The data (Fig. 1, 2, 3) showed that salt stress (EC = 10 dS m^{-1}) significantly ($p \leq 0.05$) reduced the shoot length, shoot fresh and dry weights as compared to control conditions (EC = 2 dS m^{-1}) in Auqab-2000 (salt sensitive) and SARC-5 (salt tolerant). Extent of reduction was higher in Auqab-2000 than SARC-5. Exogenous application of Si increased the shoot length, shoot fresh and dry weights under saline and non-saline conditions at all Si levels used (0, 50 and 100, 150 and 200 mg L^{-1}). However, higher values were recorded in plants exposed to higher Si levels (150 and 200 mg L^{-1}) as compared to lower levels of Si applied (0, 50 and 100 mg L^{-1}). Comparing genotypes, under control conditions, Auqab-2000 depicted better performance in comparison to SARC-5, however, SARC-5 was better under saline environment.

Table 1. Pooled ANOVA for various growth parameters of wheat plants grown in solution culture

SOV	DF	Mean square							
		Shoot length	**Shoot fresh weight**	**Shoot dry weight**	**Root length**	**Root fresh weight**	**Root dry weight**	**Root shoot ratio**	**Total weight**
Salinity	1	1936 **	3889.0 **	08.24 **	2240**	200.13 **	2.538 **	0.124 **	19.918 *
Genotypes	1	22.76 *	021.85 *	00.01 n.s	29.01*	1.07 n.s	0.020 n.s	0.007 *	0.004 n.s
Si	4	169.63 *	283.76 **	10.63 **	5.24 n.s	3.880 *	0.016 n.s	0.013 **	11.445
Sal x Var	1	102.75 **	147.54 **	06.10 **	101.09**	9.27 *	0.035 **	0.008 *	7.05 **
Sal x Si	4	5.003 *	10.95 *	00.46 *	0.15 n.s	0.141n.s	0.004 n.s	0.001 n.s	0.47 *
Var x Si	4	3.091 n.s	2.56 n.s	00.02 n.s	0.84 n.s	0.095 n.s	0.003 n.s	0.0001 n.s	0.029 n.s
Sal xVar x Si	4	6.93 *	4.700 *	00.31 *	0.24 n.s	0.23 n.s	0.006 n.s	0.003 *	0.318 *
Error	40	2.47	1.471	00.12	3.09	0.79	0.006	0.001	0.117

What does the * mean?

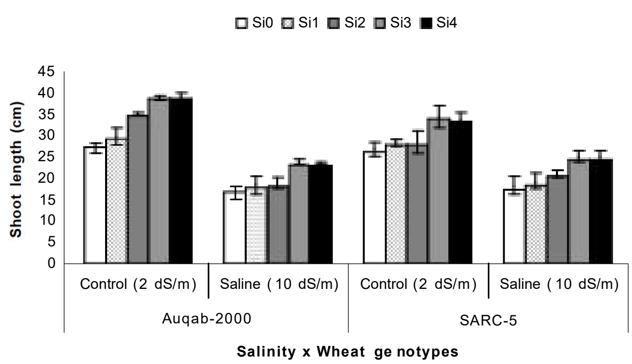

Figure 1: Effect of Si on shoot length of wheat genotypes under saline and non-saline conditions (The values are means of three replicates ± standard error (SE). Si0, Si1, Si2, Si3, Si_4 correspond to 0, 50, 100, 150 and 200 mg L^{-1} of Si, respectively)

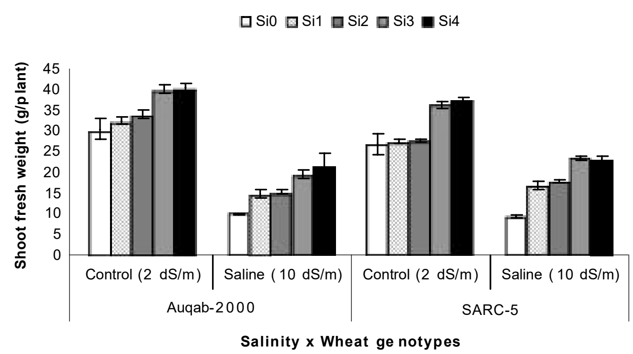

Figure 2: Effect of Si on shoot fresh weight of wheat genotypes under saline and non-saline conditions (The values are means of three replicates ± standard error (SE). Si0, Si1, Si2, Si3, Si4 correspond to 0, 50, 100, 150 and 200 mg L^{-1} of Si, respectively)

Salinity caused (Figure 4, 5, 6) a significant reduction ($p \leq 0.05$) in the root length, fresh and dry weights of wheat plants of both genotypes compared to those in non-saline solution and magnitude of decrease was less in SARC-5 as compared to Auqab-2000. The root growth inhibition caused by NaCl was not overcome by the application of calcium silicate in the solution culture. However, a non-significant gradual increase in these root growth parameters was observed with the increase in Si rate. Although, non-significant but, greater values were recorded in 150 and 200 mg L^{-1} of Si over the other degrees of Si applied. Among the genotypes, Auqab-2000 produced substantially more root length, fresh and dry weights in comparison to SARC-5 under control conditions, however, under saline environment SARC-5 proved better.

Silicon supplied wheat plants had lower root shoot ratio as compared to Si-deprived plants both under saline and non-saline conditions in both genotypes which indicates the facilitation of shoot growth over root growth (Figure 7).

The above data shows that Si has negligible effect on root but significant effect on shoot growth therefore, the recording of total seedling dry weight for deciding the optimized level of Si was focused the most one. It was calculated by pooling root and shoot dry weights as shown in Figure 8. It shows that the wheat plants were adversely affected by salt stress hence; lower weights were recorded as compared to non-saline in both genotypes. Auqab-2000 being susceptible to salinity, demonstrated more weight loss than SARC-5. With the increasing rate of Ca-silicate in saline as well as in non-saline solution culture, the plant weight continued to increase. Both 150 and 200 mg L^{-1} gave the highest values of plant weight over all other Si levels in both genotypes. These two were observed statistically at par with each other. This indicated that there was a linear increase in weight up to 150 mg L^{-1} of Si applied. Therefore, 150 mg L^{-1} level of Si was selected as an optimized level for further experiments. Furthermore, it (Fig. 13) indicated that a significant ($p \leq 0.05$) positive regression coefficient relationship ($R^2 = 0.99$, n = 4) exits between Si rate and total seedling dry weight (Figure 9).

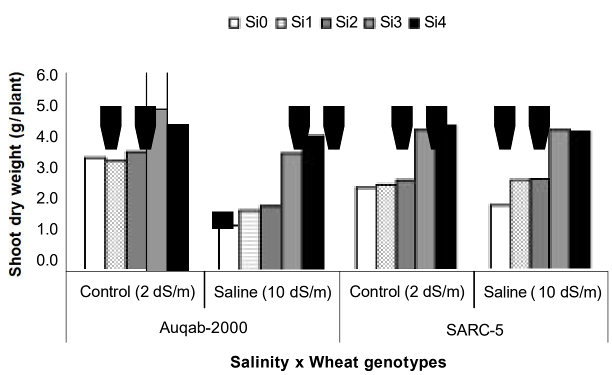

Figure 3: Effect of Si on shoot dry weight of wheat genotypes under saline and non-saline conditions (The values are means of three replicates ± standard error (SE). Si0, Si1, Si2, Si3, Si4 correspond to 0, 50, 100, 150 and 200 mg L^{-1} of Si, respectively)

Si-uptake (Figure 9) was limited by the addition of NaCl into solution culture. Salt stress reduced Si concentration in leaves of both cultivars in comparison to non-stress conditions. The Si content in flag leaves of both cultivars increased with increasing rate of Si under saline and non-saline conditions. Maximum concentration was observed in plants where Si was applied @ 150 and 200 mg L^{-1}. Minimum concentration was observed in plants where Si was not added (0 mg L^{-1}). Comparing cultivars, SARC-5 contained higher Si concentration in its flag leaves than Auqab-2000.

Sodium (Na^+) was determined to provide some insight into the mechanism of action of Si against NaCl-stress. Fig. 10 indicates the accumulation of Na^+ in flag leaf of wheat plants under saline and non-saline conditions in SARC-5 and Auqab-2000. The salt stress significantly ($p < 0.05$) increased Na^+ content in flag leaves of both cultivars in comparison to non-saline conditions. The uptake of Na^+ by Auqab-2000 was more pronounced as compared to SARC-5 under saline conditions. However, non-significant ($p > 0.05$) differences were noticed under non-saline conditions between two cultivars. Si application significantly reduced the concentration of Na^+ in flag leaves. Minimum concentration was observed in plants where Si was applied @ 150 and 200 mg L^{-1}. Maximum concentration ofNa^+ was observed in plants where Si was not added (0 mg L^{-1}).

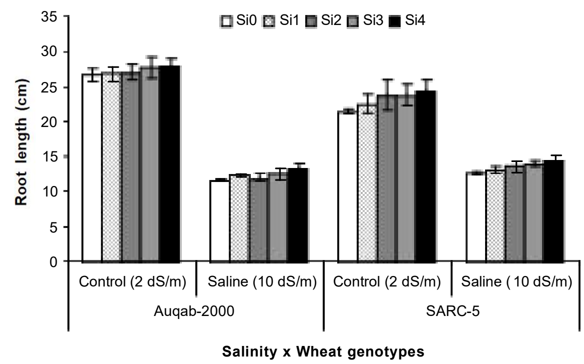

Figure 4: Effect of Si on root length of wheat genotypes under saline and non-saline conditions at $p \leq 0.05$ (The values are means of three replicates ± standard error (SE). Si0, Si1, Si2, Si3, Si4 correspond to 0, 50, 100, 150 and 200 mg L^{-1} of Si, respectively)

The data (Figure 11) showed that salt stress (10 dS m^{-1}) considerably reduced the flag leaf K^+ concentration in both cultivars in comparison to non-stress (2 dS m^{-1}) conditions. The K^+ content in flag leaves of both cultivars increased with increasing rate of Si under saline and non-saline conditions. Maximum concentration was observed in plants where Si was applied @ 150 and 200 mg L^{-1}. Minimum concentration was observed in plants where Si was not added (0 mg L^{-1}). Comparing cultivars, SARC-5 contained higher K^+ concentration in its flag leaves than Auqab-2000 under saline conditions however, the reverse was true under non-saline.

Fig. 12 indicates that when wheat plants were exposed to salt stress, K^+: Na^+ was badly affected leading towards reduced uptake of K^+ as compared to Na^+. Salt stress significantly reduced the K^+: Na^+ in both cultivars when compared with non-stress conditions. K^+: Na^+ increased with increase in exogenous Si in the order of 0 > 50 > 100 >150 > 200 mgL^{-1}under saline as well as non-saline conditions in both cultivars. The K^+: Na^+ in SARC-5 cultivar was higher under both saline and non-saline conditions, when compared with Auqab-2000.

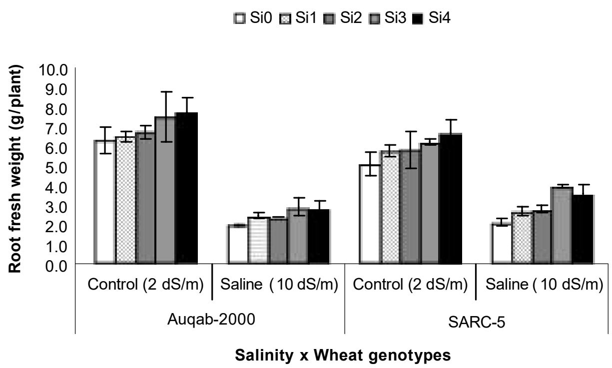

Figure 5: Effect of Si on root fresh weight of wheat genotypes under saline and non-saline conditions (The values are means of three replicates ± standard error (SE). Si0, Si1, Si2, Si3, Si4 correspond to 0, 50, 100, 150 and 200 mg L^{-1} of Si, respectively)

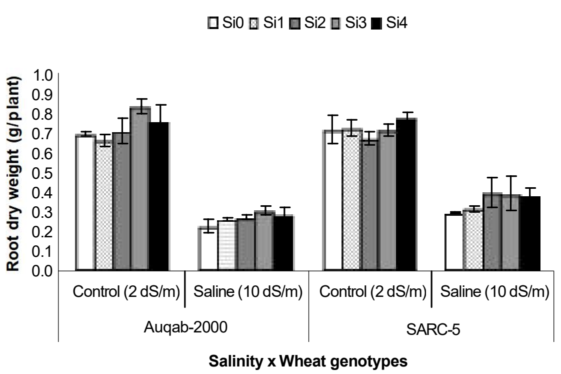

Figure 6: Effect of Si on root dry weight of wheat genotypes under saline and non-saline conditions at (The values are means of three replicates ± standard error (SE). Si0, Si1, Si2, Si3, Si4 correspond to 0, 50, 100, 150 and 200 mg L^{-1} of Si, respectively)

Discussions

Salt sensitivity of wheat plants is well known and documented like other glycophytes (Erdei *et al*., 1989; Trivedi *et al*., 1991; Zhu *et al*., 2003). Wheat plants show toxic responses to salt stress (Sharma *et al*., 2005). Si application can tolerate this stress effect (Ahmed, 1992) in wheat. Wheat has also been designated as a Si-accumulator (Mayland *et al*., 1991). Hence, the Si application significantly (Figure 9) increased the Si-content in flag leaves of wheat under saline and non-saline conditions. Silicon was deposited within the roots (Gong *et al*., 2003). It inhibited the Na^+ transportation to aerial parts of plants by its effect on transpiration movement (Yeo *et al*., 1999) or by making a complex with Na^+ (Matoh *et a1*., 1986; Ahmad *et al*., 1992). In the current study Si negatively correlated with Na^+ (Figure 14), thus it reduced the concentration of Na^+ in wheat leaves (Figure 10). Lower Na^+ is a good indicator of salt tolerance in plants.

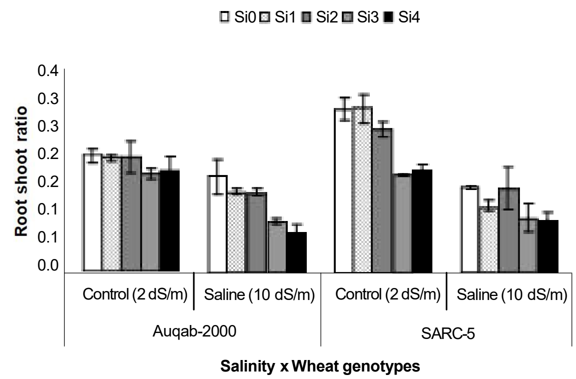

Figure 7: Effect of Si on root shoot ratio of wheat genotypes under saline and non-saline conditions at $p \leq 0.05$ (The values are means of three replicates ± standard error (SE). Si0, Si1, Si2, Si3, Si4 correspond to 0, 50, 100, 150 and 200 mg L^{-1} of Si, respectively)

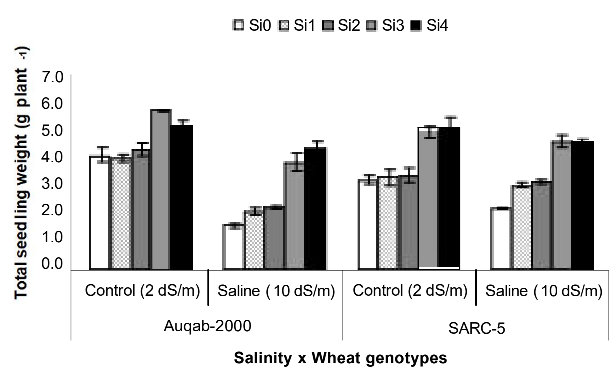

Figure 8: Effect of Si on total seedling weight of wheat genotypes under saline and non-saline conditions at $p \leq 0.05$ (The values are means of three replicates ± standard error (SE). Si0, Si1, Si2, Si3, Si4 correspond to 0, 50, 100, 150 and 200 mg L-1 of Si, respectively)

Increased K^+ concentration also shows the ability of plants to combat the salinity stress that will strongly depend upon Na^+ and Si content. The added Si increased the K^+ concentration than the plants grown without Si in saline conditions (Figure 11). Si uptake is positively correlated with K^+ and negatively with Na^+ uptake (Figure 14). Possibly, the K^+ transport was improved by Si application by its effect on the flux through K^+ ion transporters. Liang *et al.* (1999) found that the salt tolerance due to Si application is attributed to selective uptake and transport of K^+ and Na^+ by plants.

in comparison to non-saline (EC = 2 dS m^{-1}) in both wheat genotypes (Figure 1, 2, 3, 4, 5, 6). The reduced seedling growth (root and shoot growth) under salt stress might be attributed to excessive accumulation of Na^+ within plant body followed by reduction of enzymatic processes and protein synthesis (Tester & Davenport, 2003).

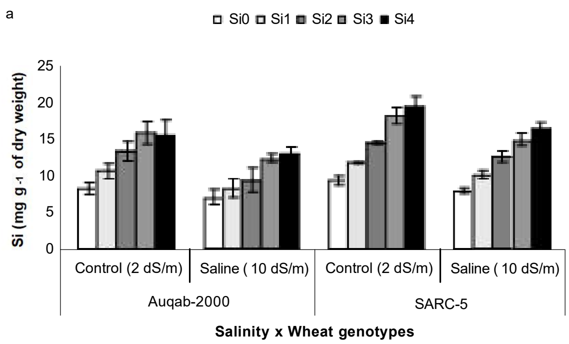

Figure 9: Effect of Si on Si concentration in flag leaves of wheat genotypes under saline and non-saline conditions (The values are means of three replicates ± standard error (SE). Si0, Si1, Si2, Si3, Si4 correspond to 0, 50, 100, 150 and 200 mg L^{-1} of Si, respectively)

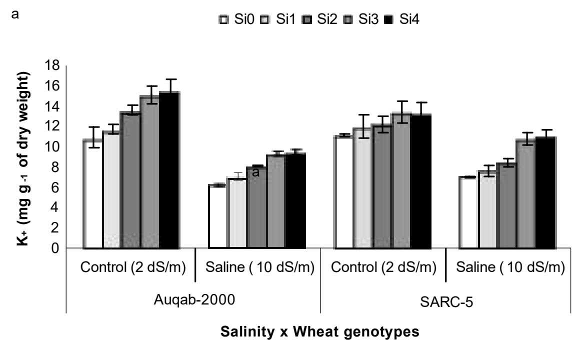

Figure 11: Effect of Si on K^+ concentration in flag leaves of wheat genotypes under saline and non-saline conditions at $p \leq 0.05$ (The values are means of three replicates ± standard error (SE). Si0, Si1, Si2, Si3, Si4 correspond to 0, 50, 100, 150 and 200 mg L^{-1} of Si, respectively)

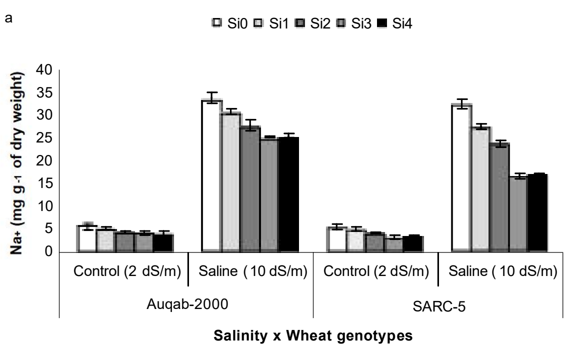

Figure 10: Effect of Si on Na^+ concentration in flag leaves of wheat genotypes under saline and non-saline conditions at (The values are means of three replicates ± standard error (SE). Si0, Si1, Si2, Si3, Si4 correspond to 0, 50, 100, 150 and 200 mg L^{-1} of Si, respectively)

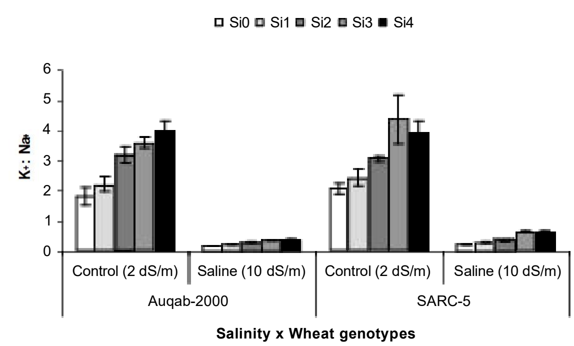

Figure 12: Effect of Si on K^+/Na^+ in flag leaves of wheat genotypes under saline and non-saline conditions at $p \leq 0.05$ (The values are means of three replicates ± standard error (SE). Si0, Si1, Si2, Si3, Si4 correspond to 0, 50, 100, 150 and 200 mg L^{-1} of Si, respectively)

Salinity ultimately influences the shoot and root growth in wheat (Ahmed *et al*., 1992), rice (Gong *et al*., 2006) and maize (Moussa, 2006) plants in the presence of NaCl added into nutrient solution. It is also evident from the current study that growth traits viz. root and shoot lengths, root and shoot fresh and dry weights were adversely influenced by salt stress when grown in saline solution (EC = 10 dS m^{-1})

Among the genotypes used in the experiment, the variety Auqab-2000 (salt sensitive) showed more susceptibility to salt stress and the reduction in root and shoot growth was more pronounced in comparison to SARC-5 (salt tolerant) under saline conditions, but, under non-saline (EC = 2 dS m^{-1}) conditions Auqab-2000 showed better results (Figure 1-8). Similar response of these genotypes to salt stress was reported by Saqib *et al*. (2004) and Tahir *et al*. (2006).

A variety of strategies are used to mitigate the adverse effects of salinity on crop plants including exogenous application of nutrients (Raza *et al*., 2006) like K^+ in wheat (Akram *et al*., 2007); N in *Phaseolus vulgaris* (Wagenet *et al*., 1983) and Ca in bean (Awada *et al*., 1995). Similarly, Si application has many more beneficial effects on plant growth and crop yields under stressful environments (Epstein, 2001; Ma *et al.,* 2001).

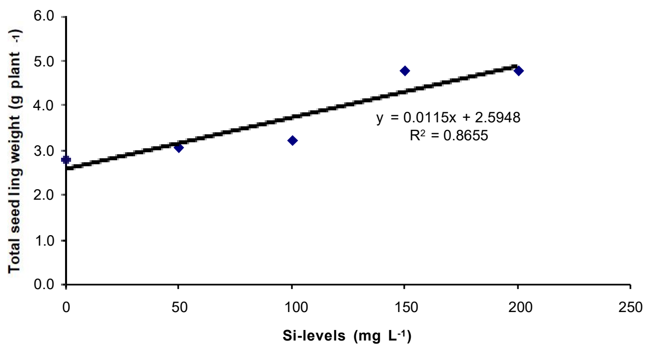

Figure 13. The linear relationship between Si-levels used and total seedling weight in wheat

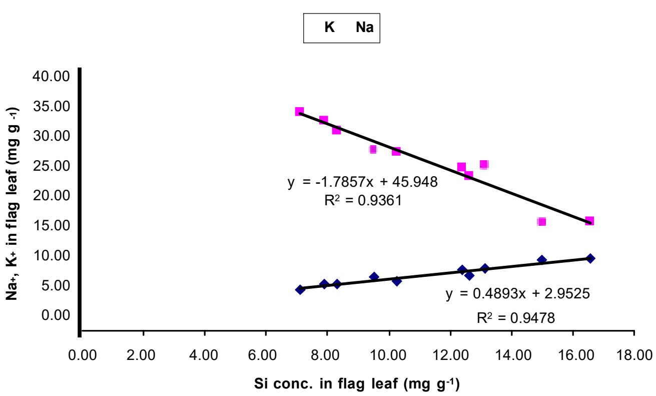

Figure 14. Correlation between Si, Na^+ and K^+ conc. in flag leaves of wheat in saline culture

The current work showed that the enhancement in shoot growth was more pronounced showing that Si application ameliorated the adverse effects of salinity by increasing root and shoot lengths and fresh and dry weights in Si-containing pots in comparison to pots where Si was not supplemented. A similar impact of Si was noticed in non-saline conditions as well (Figure 1, 2, 3, 8) indicating that it enhanced the crop growth not only under saline but also under non-saline conditions. These results are supported by Gong *et al.* (2006), who observed an enhanced shoot dry and fresh weight under no-salt stress in barley whereas Yeo *et al.* (1990) observed the similar results in rice crop only under saline conditions. It is also reported that exogenously applied Si increased the growth of a number of monocot and dicot species under salt free conditions (Adatia *et al*., 1986). Wheat growth is significantly and linearly correlated with Si application rate which indicates the concomitant increase in biomass with increasing levels of Si (Figure 9).

The possible mechanisms responsible for better crop growth in the presence of Si under stressful conditions might be the prevention of loss of water from aerial parts of plant by keeping the water status maintained by the plant (Takahashi *et al*., 1990). As a result plants maintained the photosynthetic activity to increase dry matter production (Agurie *et al*., 1992). Root shoot ratio (Figure 7) calculated on dry weight basis was lower in Si-enriched solutions as compared to Si-depleted solutions which indicated the facilitation of shoot growth over root growth and higher photosynthetic rate resulting into higher dry matter production. The current results showed that the root growth inhibition caused by NaCl was not overcome by the calcium silicate and non-significant differences were observed among different levels of Si. Si application did not increase the root length, root fresh and dry weights significantly in both genotypes under salinized and non-salinized cultures. However, non-significant increase with increase in Si was observed (Figure 1, 3, 5). Earlier, Ahmed *et al.* (1992) also reported that root growth remains unaffected in wheat by Si application. Current results are also in conformity with those of Gong *et al.* (2006) who reported that adding Si in solution culture did not alter the growth of barley root showing a little effect on root length and root dry weight (15%). Similar findings have been reported by Moussa (2006), Al-aghabary *et al.* (2004), Yeo *et al*. (1999) and Takahashi *et al*. (1990).

Conclusion

It was concluded from this experiment that the plant growth is significantly affected by salt stress in both wheat genotypes in hydroponics conditions. Silicon application significantly improved the crop growth in both the genotypes under normal as well as saline conditions indicating its importance in mineral nutrition for wheat. It occurred due to reduced Na^+ and increased K^+ uptake. Better results were obtained with the use of Si-levels (150 mg $L^{-1}$and 200 mg L^{-1}). However, these two levels were often equally effective in many parameters observed. The current results also enabled us to select the most effective (150 mg L^{-1}) level of Si out of five used against salinity.

Among the genotypes, SARC-5 showed better growth performance than Auqab-2000 under saline conditions and confirmed its salt tolerance.

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