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INCREASED GROWTH AND CHANGES IN WHEAT MINERAL COMPOSITION THROUGH CALCIUM SILICATE FERTILIZATION UNDER NORMAL AND SALINE FIELD CONDITIONS

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Salinity stress is a major and ever-present threat to crop production, especially where irrigation is necessary for agriculture. Two independent field experiments were carried out in natural non-saline (site-I; electrical conductivity [EC] < 4 dS m^{-1}) and saline (site-II; EC = 10-13.8 dS m^{-1}) fields to test the efficacy of different doses of Si (0, 75, and 150 mg kg^{-1}) on two wheat (*Triticum aestivum* L.) cultivars with different salt susceptibility, i.e., 'Auqab-2000' (salt-sensitive) and 'SARC-5' (salt-tolerant). The crop was harvested at maturity and various ionic and yield parameters were recorded. The concomitant increase in the number of tillers, number of grains per spike, grain yield, and biological yield were observed given that Si was applied under both optimal and salt-affected field conditions. It was concluded that 'SARC-5' is better than 'Auqab-2000' under salt stress. When Si was applied, similar effects were observed in both cultivars regardless of their salt sensitivity and whether the field was saline or non-saline, and it enhanced wheat growth by improving K^+:Na^+, which was adversely influenced by salt stress.

Key words: *Triticum aestivum*, salt stress, silicon, wheat growth, K^+:Na^+, water potential, stomatal conductance.

The global population of about 6.3 billion is increasing at an alarming rate. It is estimated that it will be 9.0 billion by 2050 (Lal, 2007). Efforts are underway to enhance the production of different crops to meet the food requirements of a rapidly increasing population. Salinity is one of the major factors responsible for soil degradation and low crop productivity. About one third of the world's land surface has arid or semiarid conditions (4.8×10^9 ha) of which half is estimated to be affected by salinity (Croughan and Rains, 1982) and accounts for about 7% of the world's total land area (Szaboles, 1989). Approximately 6.67 Mha of the total agricultural area in Pakistan is also affected by various degrees of salinity/sodicity (Khan, 1998).

Salinity is a major actual abiotic stress (Rueda-Puente *et al*., 2007), one of the most severe environmental problems affecting crop growth (Lopez *et al*., 2002), and along with drought, it seems to be one of the world's most serious agricultural problems. Excess of soluble salts in the 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) due to the excessive buildup of Na^+ and Cl^- (Cramer and Nowak, 1992; Lutts *et al*., 1996; Khan, 1998; Grattan and Grieve 1999). It is reported that Na^+ disturbs K^+ nutrition, which in turn inhibits enzyme activity (Jaleel *et al*., 2007). Wheat (*Triticum aestivum* L.), a glycophytic plant, is adversely affected by salinity stress (Zhu, 2003), and yield losses of up to 45% have been reported because of this (Qureshi and Barrett-Lennard, 1998).

Several chemical, physical (engineering), and biological approaches were used for better crop production in saline soils in the past. The integrated use of these approaches was crucial due to economic and environmental limitations. Genotypic variation is useful to screen and develop more salt-tolerant genotypes. Wheat genotypes also differ significantly in salinity tolerance (Munns, 2002; Flowers, 2004; Saqib *et al*., 2005) since salt-tolerant plants accumulate less Na^+ than salt-sensitive plants, which maintain the ionic balance in plant tissues (Tahir *et al*., 2006). The exogenous application of nutrients was considered as a shotgun approach to alleviate salt stress (Raza *et al*., 2006), so the damaging effects of salts were lessened with exogenously applied K^+ in wheat (Akram *et al*., 2007), N in *Phaseolus vulgaris* L. (Wagenet *et al*., 1983), and Ca in snap bean (Awada *et al*., 1995). Furthermore, some beneficial mineral nutrients have been studied that can counteract the adverse effects of salt stress such as silicon (Si), which provides significant benefits to plants at various growth stages.

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Silicon is the second most abundant element on the earth's crust after oxygen. It is accumulated in plants at a rate comparable to those of macronutrient elements such as Ca, Mg, and P (Epstein, 1999) and 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). Graminaceous plants accumulate more Si in their tissues than other species (Matichenkov and Kosobrukhov, 2004); wheat is a member of the Gramineae family and Si is designated as an accumulator so that adding Ca-silicate to salt-stressed plants can reduce their salinity stress, and it plays a multiple role in the existence of plants and crop performance. The responsible mechanism involved in salt tolerance is still not clear; however, it has been reported that Si reduces Na^+ uptake by forming a complex with Na^+ in the soil (Ahmad *et al*., 1992). Silicon is deposited in the leaves, which leads to decreased transpiration and diluted salts accumulated in the saline environment (Matoh *et al*., 1986).

The purpose of this study was to provide some additional experimental evidence about the role of Si on wheat crop biology under field conditions. To achieve this objective, the effect of Si was assessed for two cultivars with different salinity tolerances. The hypothesis was to corroborate whether Si can enhance the salt tolerance of this species under field conditions.

MATERIALS AND METHODS

Two experiments were conducted in normal and saline fields, respectively. To assess the role of Si in field level salinity tolerance, two contrasting wheat genotypes (salt-sensitive 'Auqab-2000' and salt-tolerant 'SARC-5') were grown at site-I (normal soil with EC < 4 dS m^{-1}) and site-II (naturally saline with EC = 10-13.8 dS m^{-1}). Both sites were located within a radius of less than 500 m in the Post Graduate Agricultural Research Station (PARS), University of Agriculture Faisalabad, Pakistan. The seed bed was prepared with 2-3 cultivations followed by planking. Soil samples from both sites were collected at a 0 to 15 cm depth to determine various physiochemical soil characteristics of the selected fields (Table 1). Seeds were sown at a dose of 100 kg ha^{-1} at distance of 22.5 cm between rows. Treatments mentioned below were triplicated according to a randomized complete block design with a factorial design in a 6 × 1.5 m^2 net plot size. A uniform dose of basal fertilizer was applied to all plots with 75 kg N ha^{-1} as urea, 50 kg P ha^{-1} as single super phosphate, and 30 kg K ha^{-1} as sulfate of potash (SOP). After seedling emergence (23 d after sowing), Si was applied at both sites in the respective plots at 0 (control), 75, and 150 mg Si kg^{-1} soil with calcium silicate after dissolving it with KOH at 71 °C on a hotplate. All of the Si was applied to the plots of the Si+ treatment through the placement method employing a calcium silicate solution. A $CaCl_2$ solution was applied to the Si-deficient treatment plots to balance the same total Ca as in the Si+ treatment so as to identify only the effect of Si. Observations were recorded in both field experiments.

Determination of Na^+, K^+, and Si from wheat straw

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

Silicon was determined in harvested leaves; these were oven-dried and ground into a fine powder in a Wiley mill with a built-in stainless steel chamber. The ground samples (0.5 g) were digested in 2 mL of 50% hydrogen peroxide (H_2O_2) and 4.5 g of 50% NaOH in open vessels (Teflon beakers) on a hotplate at 150 ºC for 4 h. The Si concentration was measured by the calorimetric amino molybdate blue color method (Elliot and Snyder, 1991). In a 50 mL of polypropylene volumetric flask, 1 mL of supernatant filtrate liquid was added to 10 mL of ammonium molybdate (54 g L^{-1}) solution and 25 mL of 20% acetic acid. After 5 min, 5 mL of 20% tartaric acid and 1 mL of reducing solution were added to a flask and the volume was made up of 20% citric acid. After 30 min, the absorbance was measured at the 650 nm wavelength with a UV visible spectrophotometer (Spectronic 100, Shimadzu, Kyoto, 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 Snyder, 1991).

Yield and yield components

The number of tillers per m^2 was counted, recorded, and converted into the total number of tillers per plot. Ten spikes were randomly selected from each subplot; the mean spike length, number of spikelets per spike, number of grains per spike, and 1000 grain weight were recorded. Central rows from each subplot were harvested, tied into bundles left to dry in the sun. The samples were then weighed with a spring balance and yield was converted into kg ha^{-1}. Grain yield was measured at harvest from the central rows of each subplot and yield converted into kg ha^{-1}. Finally, the harvest index (HI) for each plot was calculated with the following formula:

$$HI = (\text{Grain yield}/\text{biological yield}) \times 100$$

Data collected during the study were statistically analyzed by Fisher's ANOVA Technique and significant means were separated by the Least Significant Difference (LSD) Test at P = 0.05 (Steel and Torrie, 1996).

RESULTS

Data regarding plant height, number of tillers, number of spikelets per spike, number of grains per spike influenced by salinity and Si are shown in Table 2. Salinity stress

Table 1. Soil properties of Experimental Field at Faisalabad, Pakistan.

Property	Site-I (normal field)	Site-II (natural saline field)
Sand, %	62	56
Silt, %	24	25
Clay, %	17	21
Textural class	Sandy loam	Sandy clay loam
pH	7.25	8.3
Electrical conductivity, dS m^{-1}	1.28	10 – 13.8
Organic matter, %	0.91	0.79
Calcium carbonate, %	2.20	1.99
Olsen-P, mg kg^{-1}	4.80	6.04
Available K, mg kg^{-1}	168	218
Available Si, mg kg^{-1}	39	35

significantly ($P \leq 0.05$) reduced these parameters in the saline field (site-II) when compared with the normal field (site-I). Calcium silicate fertilization increased values significantly by 0 < 75 < 150 mg kg^{-1} in both saline and non-saline field conditions. Comparing the cultivars for plant height, 'Auqab-2000' (salt-sensitive) was higher than 'SARC-5' (salt-tolerant) at both site-I and site-II.

Table 2 indicates that spike length and grain weight were lower at site-II (saline field) than at site-I. Applying Ca-silicate did not influence spike length and grain weight of wheat plants at site-I resulting in non-significant differences among all Si levels; nonetheless, applying Si at site-II improved the unpleasant effect of salinity and caused a significant increase in spike length and grain weight values in both genotypes. The 'Auqab-2000' cultivar had higher spike length than 'SARC-5' at site-I and the contrary was true for site-II.

Biological and grain yield data at site-I and site-II influenced by Si and salinity are shown in Table 2. Dry matter and wheat grain production of in both cultivars was negatively affected at site-II by salinity stress when compared with site-I. Calcium silicate fertilization remarkably improved and enhanced yield at site-I and site-II in both cultivars with the increasing rate of Si, i.e., 0, 75, and 150 mg kg^{-1}. At site-I, 'Auqab-2000' performed better and had a higher yield than 'SARC-5' and the contrary was true for site-II. It is evident that as the harvest index value increases, the plant's physiological efficiency to convert dry matter into grain yield is higher (Table 2). Fertilization of saline and non-saline fields with Ca-silicate improved the harvest index in both cultivars. 'SARC-5'shad a higher harvest index than 'Auqab-2000' in the saline field. Table 3 shows a higher concentration of Na^+ in wheat straw grown in a saline field than in a non-saline field. Applying Si significantly lowered Na^+ concentration in wheat straw at both sites. The reduction in Na^+ content in the flag leaf occurred by 0 > 75 > 150 mg kg^{-1} at both sites. Site-I had a higher Na^+ content reduction than site-II. 'SARC-5' contained less Na^+ than 'Auqab-2000' at both sites. Table 3 shows that on the average lower values of K^+ and Si concentrations were observed at site-II than site-I in both cultivars. Substantial increases in K^+ and Si concentrations in wheat straw were noted when Si was applied in both cultivars grown at site-I and site-II. At both sites, the highest K^+ and Si contents were observed in the wheat plot fertilized with Si at 150 mg kg^{-1} and the lowest where it was not applied. 'SARC-5' contained a significantly lower K content than 'Auqab-2000' at site-I and the contrary is true for site-II, while Si concentration was higher in 'SARC-5' at both sites. Figure 1 shows that higher values of K^+:Na^+ were observed at site-II than site-I in both cultivars. At site-II, 'Auqab-2000' had a lower value than 'Sarc-5'. A considerable increase of K^+:Na^+ in wheat straw was noted when Si was applied in both cultivars grown at site-I and site-II. 'SARC-5' contained a significantly higher K^+:Na^+ content than 'Auqab-2000' at both sites.

DISCUSSION

Salinity stress limits crop yield mostly in arid and semiarid regions of the world (Munns, 2005). Wheat is

Table 2. Effect of Si on yield and yield components of wheat cultivars under both saline and non-saline conditions.

		Silicon level					
		Control Si0		Si1		Si2	
Parameters	Genotype	Site-I (Non saline)	Site-II (Saline)	Site-I (Non saline)	Site-II (Saline)	Site-I (Non saline)	Site-II (Saline)
Plant height, cm	Auqab-2000	69.95c	41.36cd	71.16c	43.53b	76.73a	46.94b
	SARC-5	65.75d	36.72e	64.23d	39.68d	73.96a	42.90bc
Number of tillers m^{-2}	Auqab-2000	426b	336c	421.66b	344c	454.33a	357.33a
	SARC-5	408.67c	341.66bc	405.33c	443b	426.33b	354.00a
Spike length, cm	Auqab-2000	10.43a	7.05b	10.48a	7.66bc	10.52a	8.68a
	SARC-5	8.84b	7.22c	8.83c	7.31c	8.84b	8.28ab
Number of spikelets per spike	Auqab-2000	16.66bc	9.66e	17.66ab	10.66d	18.33a	12.33c
	SARC-5	14.66d	13.00c	15.66cd	14.33b	16.33c	15.66a
Number of grains per spike	Auqab-2000	40.00d	27.77d	42.33c	30c	45.00ab	33.33b
	SARC-5	39.66d	29.00cd	43.33bc	33b	45.66a	36.66a
1000 grain weight, g	Auqab-2000	39.86a	28.36d	41.15a	31.40c	41.23a	35.16ab
	SARC-5	31.90b	28.27d	32.35b	34.26c	32.95b	36.16a
Biological yield, kg ha^{-1}	Auqab-2000	7590.83d	4505d	8340.00c	4886.66c	9825.83a	5408.33c
	SARC-5	6654.16f	4630d	7313.33e	5054.16c	8602.50b	5785.83a
Grain yield, kg ha^{-1}	Auqab-2000	2810c	1560e	3275.83b	1814.16cd	3822.50a	2067.50b
	SARC-5	2465.83d	1670de	2749.16c	1882.50c	3230.83b	2244.16a
Harvest index	Auqab-2000	0.37a	0.34b	0.39a	0.37ab	0.38a	0.38a
	SARC-5	0.37a	0.36ab	0.37a	0.37ab	0.37a	0.38a

Si0, Si1, and Si2 represent 0, 75, and 150 mg Si kg^{-1}, respectively. The values are means of three replicates. Means with the same letter do not differ at P = 0.05.

Table 3. Effect of Si on ionic composition of wheat cultivars under both saline and non-saline conditions.

		Silicon level					
		Control Si0		Si1		Si2	
Parameters	Genotype	Site-I (Non saline)	Site-II (Saline)	Site-I (Non saline)	Site-II (Saline)	Site-I (Non saline)	Site-II (Saline)
Na^+, mg g^{-1} dry weight	Auqab-2000	5.45a	21.32a	5.26a	16.72c	5.14a	9.72e
	SARC-5	5.42a	17.37b	4.63b	13.58c	4.38b	8.46f
K^+, mg g^{-1} dry weight	Auqab-2000	10.47e	9.52e	15.39c	11.73d	22.42a	12.52c
	SARC-5	9.43f	12.69c	13.56d	13.66b	19.37b	14.49a
Si, mg g^{-1} dry weight	Auqab-2000	11.52f	9.18e	16.66d	9.36e	22.50b	10.25d
	SARC-5	12.39e	11.20c	18.60c	16.51b	24.60a	20.56a
K^+:Na^+	Auqab-2000	1.92c	0.44e	2.92b	0.70d	4.35a	1.28b
	SARC-5	1.74c	0.73d	2.92b	1.00c	4.43a	1.73a

Si0, Si1, and Si2 represent 0, 75, and 150 mg Si kg-1, respectively. The values are means of three replicates. Means with the same letter do not differ at P = 0.05.

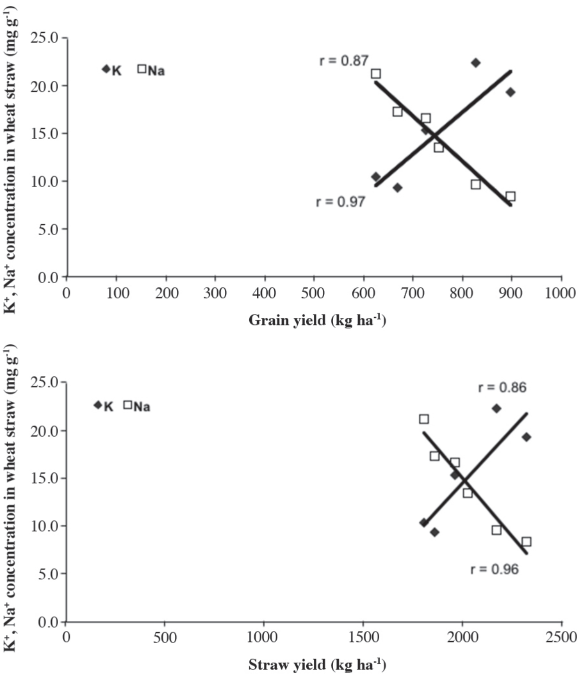

Figure 1. A schematic diagram showing the correlation between K^+ and Na^+ and grain yield and straw yield under saline field conditions.

a frequent crop in these regions; therefore, its growth and yield is severely affected by salt stress. In the present field study, a lower yield and yield components of both cultivars were observed in a saline field as compared to a non-saline field. The reduced crop growth and yield was attributed to excessive accumulation of soluble salts in the root zone (Grattan and Grieve, 1999; Munns, 2005; Tahir *et al*., 2006).

It is reported 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). Silicon is known to improve the growth of plants subjected to salinity stress (Liang *et al*., 1996). A similar positive effect of Si was observed in the current study, thus indicating that when fields were fertilized with Ca-silicate, both biological and grain yield increased in both saline and non-saline fields (Table 2).

Biological yield is the mutual contribution of yield components such as the number of tillers per unit area, plant height, number of grains per spike, and grain weight. Any factor causing change in these components will reflect change in biological yield. Moreover, the efficiency and effectiveness of any technology package is ultimately reflected by the level of grain yield, which can also be described as a function of the cumulative behavior of yield components. Applying Ca-silicate resulted in a significant increase in yield and yield-contributing factors in both saline and non-saline fields (Table 2). The current findings were also supported by Ando *et al*. (1999), who indicated an increase in dry matter yield with an increasing rate of Si nutrition in a rice field. The increased rice yield was attributed to the increased number of grains per unit area and the percentage of mature grains through Si supplementation. Daren *et al*. (1994) also studied 10 rice cultivars grown at two different sites in Florida on Si-deficient organic Histosol and indicated a higher yield as a result of an increase in the number of grains per panicle due to applying Si, whereas 100 seed weight and panicles per m^2 exhibited less change. In this study, the increase in wheat yield might be due to increased water status and a higher photosynthetic rate as suggested by Hattori *et al*. (2005) for *Sorghum bicolor*. Comparing the cultivars on the basis of yield and its components, it was observed that 'Auqab-2000' (salt-sensitive) performed better in a non-saline field and had a higher biological yield, grain yield, harvest index, number of tillers, spike length, number of grains, 1000 grain weight, and number of spikelets per spike when compared to 'SARC-5' at all Si levels and the contrary was true in a saline field (Table 2).

Silicon deposited in plants helped to maintain a balanced and efficient absorption and translocation of mineral elements required for better growth (Islam and Saha, 1969; Marschner *et al*., 1990). Silicon also helps in competing with saline conditions by reducing Na^+ toxicity while maintaining higher rates of K^+ uptake, which is one of the major reasons for salt tolerance in wheat suggested by Ahmad *et al*. (1992) and Tahir *et al*. (2006).

The current research (Table 3) showed that Si content in the plant body of both cultivars increased as Si increased under both saline and non-saline conditions. It is evident that Si deposition improved the toxic effects of salinity by enhancing K^+ uptake as compared to the Na^+ ion in saline and non-saline fields (Table 3). This might be due to the immobilization of toxic Na^+ that occurred because Si was deposited in the root exodermis and endodermis (Gong *et al*., 2006). Liang *et al*. (2005) suggested a significant increase in K^+ uptake and a decrease in Na^+ uptake in barley (*Hordeum vulgare* L.) under salt stress due to the increased activity of the plasma membrane H^+-ATPase. For wheat crops, Ahmad *et al*. (1992) reported the binding of soluble Si with Na^+ in the roots, thus retarding its movement to the plant's aerial parts, which significantly decreased Na^+ contents in flag leaves and roots of salt-stressed wheat plants.

It can be suggested that growth enhancement is associated with improved K^+:Na^+ as an indicator of salt tolerance (Figure 1) and shown by a positive relationship between grain yield and K^+ and a negative relationship with Na^+. Similarly, straw yield was positively correlated with K^+ and negatively correlated with Na^+. On the basis of ionic status change, it was observed that in the saline field, 'SARC-5' absorbed less Na^+ than 'Auqab-2000' at all applied Si levels; therefore, 'SARC-5' is a higher accumulator of Si and K^+ than salt-sensitive cultivars when fertilized with Si under both saline and non-saline conditions.

CONCLUSIONS

It was concluded from the current field study that using Si is beneficial to mitigate salinity stress under a wide range of field conditions. The improvement in crop growth was associated with reduced Na^+ uptake and increased K^+ uptake resulting in an improved K^+:Na^+ ratio; 'SARC-5' performed better than 'Auqab-2000' under salt stress.

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Aumento de crecimiento y cambios en la composición mineral de trigo por fertilización con silicato de calcio bajo condiciones de campo normales y afectadas por sales. El estrés salino es un riesgo importante y siempre presente para la producción de cultivos, especialmente donde el riego es una inevitable ayuda a la agricultura. Se realizaron dos experimentos de campo independientes en campos no salino natural (sitio I; conductividad eléctrica [EC] < 4 dS m^{-1}) y salino (sitio II; EC = 10-13.8 dS m^{-1}) para probar la eficacia de diferentes dosis de Si (0, 75, y 150 mg kg^{-1}) en dos cultivares de trigo (*Triticum aestivum* L.) difiriendo en susceptibilidad a sales: 'Auqab-2000' (sensible a sales) y 'SARC-5' (tolerante a sales). El cultivo se cosechó a la madurez y se registraron varios parámetros iónicos y de rendimiento. Se observó que el aumento concomitante en número de macollas, número de granos por espiga, rendimiento de grano, y rendimiento biológico se debió a aplicación de Si bajo condiciones de campo óptimas y afectadas por sales. Se concluyó que 'SARC-5' rinde mejor que 'Auqab-2000' bajo condiciones de estrés salino. Efectos casi similares por aplicación de Si se observaron para ambos cultivares independiente de la sensibilidad a sales y su inclusión en el campo ya sea salino o no salino aumentó el crecimiento del trigo mejorando la relación K^+:Na^+ adversamente influenciada por el estrés salino.

Palabras clave: *Triticum aestivum*, estrés salino, silicio, crecimiento de trigo, K^+:Na^+, potencial hídrico, conductancia estomática.

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