African Journal of Biotechnology Vol. 11(3), pp. 606-615, 10 January, 2012
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB11.1757
ISSN 1684–5315 © 2012 Academic Journals

Full Length Research Paper

Silicon mediated biochemical changes in wheat under salinized and non-salinzed solution cultures

Anser Ali[1]*, Shahzad M. A. Basra[2], Javaid Iqbal[1], Safdar Hussain[1], M. N. Subhani[1], Muhammad Sarwar[1] and Ahmad Haji[3]

[1]University of Agriculture, Faisalabad-38040, Pakistan.
[2]Department of Crop Physiology, University of Agriculture, Faisalabad-38040, Pakistan.
[3]Agriculture Adaptive Research Complex, Dera Ghazi Khan, Pakistan.

Accepted 9 December, 2011

Silicon (Si) can alleviate salinity damage, a major threat to agriculture that causes instability in wheat production. We report on the effects of silicon (150 mg L^{-1}) on the morphological, physiological and biochemical traits in wheat (*Triticum aestivum* L.) cultivars (salt sensitive; Auqab-2000 and salt tolerant; SARC-5) differing in salt tolerance under saline (10 dS m^{-1}) and non-saline (2 dS m^{-1}) hydroponic culture. Silicon supplementation into the solution culture improved wheat growth and K^+:Na^+ with reduced Na^+ and increased K^+ uptake. Moreover, higher relative water content (RWC), increase in chlorophyll fractions and its ratios and stimulated activities of superoxide dismutase (SOD) and catalase (CAT) were observed. Nevertheless, the activity of peroxidase (POD) was reduced. We conclude that silicon inclusion into the growth medium is of assistance for wheat growth by maintaining plant water status, better K^+:Na^+, low electrolyte leakage and improved plant defense system adversely influenced by salt stress. SARC-5 showed better performance than Auqab-2000.

Key words: Antioxidants, K^+:Na^+, silicon, salt stress, wheat growth.

INTRODUCTION

Salinity, a major abiotic stress at present (Rueda-Puente et al., 2007) and the response of plants to excessive salinity is multifaceted and involves changes in plant's morphology, physiology and metabolism (Hilal et al., 1998; Rhoades, 1993), ultimately diminishing growth and yield (Ashraf and Harris, 2004) through osmotic effects, nutritional imbalances and specific ion toxicities (Grattan and Grieve, 1999; Munns, 2005; Tahir et al., 2006). Salts present in the soil solution exert an osmotic pressure and reduce the soil water potential making water unavailable to plants as reported by Munns et al. (2006). Ionic imbalance and specific ion toxicity (primary cause of growth reduction) takes place in the cells due to excessive buildup of Na^+ and Cl^- which affect the uptake of other mineral nutrients (Cramer and Nowak, 1992; Khan et al., 1998; Grattan and Grieve, 1999; Chinnusamy et al., 2005) like K^+ nutrition and inhibit the activities of many enzymes (Jaleel et al., 2007). Salt stress not only imposes the osmotic stress and ion toxicity, but also marked as an oxidative stress (Guetadahan et al., 1998) which can stimulate the accumulation of reactive oxygen species (ROS) such as superoxide, hydrogen peroxide, hydroxyl radical and singlet oxygen (Lee et al., 2001) which attack nucleic acids, proteins and lipids (Menezes-

*Corresponding author: E-mail: uafanser@gmail.com. Tel: 00923017175236.

Abbreviations: RWC, Relative water content; **SOD,** superoxide dismutase; **CAT,** catalase; **POD,** peroxidase.

Benavente et al., 2004) elevating membrane permeability (Tabaei-Aghdaei et al., 2000) and show reduction in chlorophyll contents (Dhindsa et al., 1981). Silicon (Si) is the 2nd most abundant element on the earth crust after oxygen. Si application can moderate the salinity stress in plants and plays a multitude role in plant existence and crop performance (Ahmed et al., 1992). A number of possible mechanisms are reported through which Si may increase salinity tolerance in plants (Liang et al., 2007) including increased plant water status (Romero et al., 2006), enhanced photosynthetic activity and maintenance of ultra structure of leaf organelles (Shu and Liu, 2001), stimulation of ROS scavenging system (Zhu et al., 2004), immobilization of toxic Na^+ ion (Liang et al., 2003) due to its complexation with Si (Ahmed et al., 1992), reduced Na^+ uptake in plants and enhanced K^+ uptake (Yeo et al., 1999; Liang et al., 2005; Tahir et al., 2006), higher K^+:Na^+ selectivity (Hasegawa et al., 2000). Adding Ca-silicate in salinity-stressed plants maintains membrane permeability, chlorophyll content, stomatal conductance, transpiration, net photosynthesis, intercellular CO_2 (Murillo-Amador, 2007) by diluting salts accumulated in saline environment (Matoh et al., 1986). Graminaceous plants accumulate more Si in their tissues than other species (Matichenkov and Kosobrukhov, 2004). Wheat is a member of gramineae family designated as Si accumulator is adversely affected by salinity stress (Zhu, 2003) showing yield losses up to 45% (Qureshi and Barrett-Lennard, 1998); however, the variation exists in salt tolerance of wheat genotypes (Munns, 2002; Flowers, 2003; Saqib et al., 2005). In terms of effect of Si on defense system of wheat, information is lacking.

The purpose of the current study was to explore the antioxidative role of Si in wheat crop under saline environment and present some experimental evidence about its significance in crop biology. The hypothesis was to verify whether Si may be useful to enhance the salt tolerance of this species via bringing a change in morphological, physiological and biochemical traits. In view of the aforementioned considerations, the current study was accomplished with the objectives: (I) to study the variation in salinity tolerance of two contrasting wheat cultivars and, (II) to study the physiological and biochemical insight of wheat in response to Si applied under salinity stress.

MATERIALS AND METHODS

Plant material and experimental conditions

The solution culture experiment was conducted under saline (10 dS m^{-1}) and non-saline (2 dS m^{-1}) hydroponics conditions in the wheat cvs (salt sensitive; Auqab-2000 and salt tolerant; SARC-5) in response to Si application (Si+ = 150 mg L^{-1}) against control (Si- = 0 mg L^{-1}) under rain protected net house conditions at the Department of Crop Physiology, University of Agriculture, Faisalabad; Pakistan. The whole Si was applied to the pots of the Si+ treatment at 150 mg L^{-1} using calcium silicate solution. $CaCl_2$ solution was applied to the pots of the Si-deficient treatment to balance the same total of Ca as in the Si+ treatment to know the sole effect of Si. Average temperatures in the net house were 20 ± 7°C during the day and 12 ± 5°C 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. Wheat seeds were grown in sand taken in plastic trays and irrigated with distilled water. Two weeks after sowing, 15 uniform size seedlings were transferred to plastic tubs having continuously aerated half strength Johnsons' 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. Completely randomized design with three replicates in factorial arrangement was followed. Hydrogen ion activity (pH) of the solution was monitored and adjusted daily at 6.5 to 5.5 and solution was changed weekly. Various growth, physiological and analytical parameters were recorded after 32 days of transplanting by harvesting and thoroughly washing the flag leaves of wheat plants.

The observations were recorded according to standard procedures given as follows:

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

The oven dried and grinded 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).

Determination of Si from flag leaf

The leaves of harvested plants were oven dried and grinded in a Wiley mill built-in with stainless steel chamber into fine powder. The grinded samples (0.5 g) 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°C for 4 h. Si concentration was measured using calorimetric amino molybdate blue color method (Elliot and Synder, 1991). To 1 ml of supernatant filtrate liquid, 10 ml of ammonium moblybdate (54 g L^{-1}) solution and 25 ml of 20% acetic acid was added in 50 ml of polypropylene volumetric flask. After 5 min, 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 min, 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).

Chlorophyll contents

The chlorophylls a and b were determined according to the method of Arnon (1949). Fresh leaves (0.2 g) were cut and extracted overnight with 80% acetone at 0 to 4°C. The extracts were centrifuged at 10,000 x g for 5 min. Absorbance of the supernatant was read at 645, 663 and 480 nm using a spectrophotometer (Hitachi-U2001, Tokyo, Japan). The chlorophylls a and b were calculated by the following formulae:

Chl a = [12.7 (OD 663) -2.69 (OD 645)] x V/1000 x W
Chl b = [22.9 (OD 645) -4.68 (OD 663)] x V/1000 x W

Where, V is the volume of the extract (ml) and W is the weight of the fresh leaf tissue (g).

Relative water content

A sample consisting of 5 flag leaves was taken from each pot. Fresh weight of each sample was measured. Leaves were soaked in distilled water for 14 to 16 h. After soaking period, the leaves were wiped with tissue paper and soaked weight was measured. Afterwards, samples were oven dried at 80°C to determine dry weight for each sample. For each pot, relative water content was calculated by using the formula proposed by Turner (1986) given as follows:

$$RWC = \frac{\text{Fresh weight - Dry weight}}{\text{Soaked weight - Dry weight}}$$

Quantitative estimation of proteins

To determine protein content, the frozen grinded plant material was suspended in lysis buffer containing 0.05 M Tris-HCl, pH 7.5, 0.025 M EDTA, and 1% sodium dodecyl sulfate and incubated at 25°C for 30 min. For more complete cell lysis, the procedure of freezing and thawing was repeated twice and then the lysate was centrifuged in microcentrifuge machine (Eppendorf 5415c) at 12,000 x g for 5 min at room temperature. Supernatant was used for protein estimation. Quantitative protein estimation in leaves was performed by the method of Bradford (1976) using Bovine Serum Albumin as standard. Different dilutions (10 to 100 µg/100 µl) of bovine serum albumin from stock solution were prepared in potassium phosphate buffer (50 mM, pH 7.0) to make standards. 100 µl of supernatant was mixed with 1.9 ml of dye reagent and the mixture was left for 5 min to form a protein dye complex. Then the absorbance was measured at 595 nm by using spectrophotometer (spectronic 21 D, Milton Roy). A standard curve was prepared by plotting the log of concentration on x-axis against their absorbance at 595 nm on y-axis. Protein concentration (mg/g fresh weight) was calculated using the following linear regression equation:

$y = 0.8658x + 0.5041$

Activities of antioxidant enzymes

Enzyme extraction

For the extraction of antioxidant enzymes, fresh leaves (0.5 g) were ground in pestle and mortar using 50 mM cooled phosphate buffer (pH 7.8). After filtration through cheese cloth, the homogenate was centrifuged at 15000 x g for 20 min at 4°C and the supernatant was used for enzymes assays.

Superoxide dismutase (SOD)

The activity of SOD was assayed following the method of Giannopolitis and Ries (1977) by monitoring the inhibition of photochemical reduction of nitroblue tetrazolium (NBT) at 560 nm. The activity of SOD was determined by adding 50 µl of the enzymatic extract to a solution containing (total reaction solution including enzyme extract of 3 ml) 50 µM NBT (NBT dissolved in ethanol), 1.3 µM riboflavin, 13 mM methionine, 75 nM EDTA and 50 mM phosphate buffer (pH7.8). The reaction solutions were kept in a chamber under illumination of fluorescent lamps of 30 W. The reaction was started by turning the fluorescent lamps on and stopped 5 min later by turning them off. The blue formazane produced by NBT photoreduction was measured as increase in absorbance at 560 nm. The reaction mixture without leaf extract was taken as control and kept in light. However, blank solution having the same complete reaction mixture (including enzyme extract) was kept in the dark. The absorbance of the irradiated solution at 560 nm was read using a UV-visible spectrophotometer (IRMECO U2020). One unit of SOD was defined as the amount of enzyme required to cause 50% inhi-bition of the rate of NBT reduction at 560 nm in comparison with tubes lacking the plant extract.

Catalase (CAT) and peroxidase (POD)

Activities of CAT and POD were assayed following Chance and Maehly (1955) with some modification. The final volume of the reaction mixture for CAT (3 ml) contained 50 mM phosphate buffer (pH 7.0), 5.9 mM H_2O_2 and 0.1 ml enzyme extract. The reaction was initiated by adding 100 µl of the leaf crude extract (enzyme extract) to the reaction mixture. Changes in absorbance of the reaction solution due to decomposition of H_2O_2 at 240 nm were read every 20 s using a UV-visible spectrophotometer (IRMECO U2020). CAT activity was expressed as units (µmol of H_2O_2 decomposed per min) per mg of protein. One unit CAT activity was defined as an absorbance change of 0.01 units per min. The activity of POD was determined by guaiacol oxidation method. The final volume of the reaction mixture for POD (3 ml) contained 50 mM phosphate buffer (pH 7.0), 20 mM guaiacol, 40 mM H_2O_2 and 0.1 ml enzyme extract. Changes in absorbance of the reaction solution at 470 nm were determined every 20 s. 1 unit POD activity was defined as the change of 0.01 absorbance unit per min per mg of protein.

Electrolyte leakage

Electrolyte leakage (EL) was calculated to appraise membrane permeability. This method is based on Lutts et al. (1999). EL was measured with electrical conductivity meter with Mi-70 Benchmeter EC/TDS/NaCl/temperature. Fully expanded leaves from plants were cut into 1 cm^2 segments. Leaf samples were then put into individually stoppered vials containing 10 ml distilled water. These samples were incubated at room temperature (25ºC) on a shaker (100 rpm) for 24 h. Electrical conductivity of bathing solution (EC_1) was determined. The same samples were then placed in an autoclave at 120°C for 20 min and the second reading (EC_2) was determined after cooling to room temperature. The EL was calculated as EC_1/EC_2 and expressed on percentage basis.

Determination of growth

Plants were harvested and left for sun drying. After that samples were oven dried till constant weight and weighed with spring balance and yield per plant was recorded.

Table 1. Effect of Si on Chl a, b, a + b, a/b, electrolyte leakage leaf area, relative water content, dry matter yield of wheat cultivars both under saline and non-saline conditions.

Parameter	Genotype	Control (2 dS/m)		Saline (10 dS/m)	
		Si-	Si+	Si-	Si+
Chlorophyll a (µg g^{-1} of fresh weight)	Auqab-2000	1.85^{a}	1.86^{a}	1.10^{e}	1.24^{d}
	SARC-5	1.76^{ab}	1.77^{b}	1.25^{d}	1.37^{c}
Chlorophyll b (µg g^{-1} of fresh weight)	Auqab-2000	0.74^{a}	0.76^{a}	0.59^{c}	0.61^{c}
	SARC-5	0.76^{ab}	0.77^{a}	0.63^{bc}	0.65^{c}
Chlorophyll a/b ratio	Auqab-2000	2.51^{a}	2.48^{a}	1.87^{cd}	2.03^{bcd}
	SARC-5	2.32^{ab}	2.29^{abc}	1.98^{d}	2.12^{bcd}
Chlorophyll a + b	Auqab-2000	2.59^{a}	2.62^{a}	1.69^{d}	1.86^{c}
	SARC-5	2.52^{a}	2.54^{a}	1.89^{c}	2.02^{b}
Electrolyte leakage	Auqab-2000	16.87^{bc}	14.13^{de}	19.04^{a}	15.64^{cd}
	SARC-5	15.42^{cd}	13.00^{e}	18.07^{ab}	10.86f
Relative water content	Auqab-2000	87.96^{a}	88.83^{a}	73.28^{d}	80.49^{c}
	SARC-5	87.49^{ab}	89.67^{a}	74.76^{d}	84.81^{b}
Dry matter yield (g/plant)	Auqab-2000	5.12	6.15	2.57	4.86
	SARC-5	4.21	5.94	3.23	5.63

$p \leq 0.05$ (the values are means of three replicates). Si- and Si+ represent 0 and 150 mg kg^{-1} of Si respectively.

RESULTS

Effect of Si application on mineral contents (K^+, Na^+, K^+:Na^+ and Si content) in wheat

Sodium (Na^+) was determined to provide some insight into the mechanism of action of Si against NaCl-stress. Table 2 indicates the significant ($p < 0.05$) accumulation of Na^+ in flag leaf of wheat plants under saline conditions (10 dS m^{-1}) in SARC-5 and Auqab-2000 in comparison to non-saline (2 dS m^{-1}). The uptake of Na^+ by Auqab-2000 was more pronounced and significant as compared to SARC-5 under saline conditions. Si application significantly ($p < 0.05$) reduced the concentration of Na^+ in flag leaves in both cultivars under saline and non-saline conditions. The data (Table 2) showed that salt stress (10 dS m^{-1}) considerably reduced the flag leaf K^+, K^+:Na^+ and Si concentration in both cultivars in comparison to non-stress (2 dS m^{-1}) conditions. The addition of Si (150 mg L^{-1}) significantly ($p < 0.05$) increased the K^+, K^+:Na^+ and Si concentration in plants than those grown without Si both under normal and saline conditions in both cultivars. Comparing cultivars, lesser K^+ and K^+:Na^+ was observed in Auqab-2000 (salt sensitive) in comparison to SARC-5 (salt tolerant) under non-saline conditions, but, under saline conditions, higher K^+ content and K^+:Na^+ was observed in SARC-5 than Auqab-2000.

Effect of Si application on water status, chlorophyll fractions and electrolyte leakage in wheat

Salinity (Table 1) significantly lowered the relative water content (RWC), Chl a, b, a + b and a/b in both wheat cultivars as against non-saline conditions. Added Si did not enhance the RWC, Chl a, b, a + b and a/b obviously in both cultivars in comparison to the treatment where Si was not added under non-saline conditions. However, under stress conditions, Si application improved all these RWC, Chl a, b, a + b and a/b to a significant extent in both cultivars in comparison to Si-deprived. Among cultivars, Auqab-2000 depicted better performance in comparison to SARC-5 under control conditions, but under saline environments SARC-5 exhibited higher values. Electrolyte leakage (EL) was measured to identify the membrane stability or permeability. Added NaCl into the solution culture impaired the membrane stability and significantly increased the EL in both cultivars in comparison to the treatment, where salt was not added. Inclusion of Ca-silicate into the solution culture significantly decreased EL under saline and non-saline conditions in both the cultivars in comparison to the solution where Si was not added (Table 1). Among cultivars, Auqab-2000 showed higher values of EL indicating more susceptibility as compared to SARC-5 both under saline and non-saline conditions (Table 1).

Table 2. Effect of Si on soluble proteins, catalase, peroxidase, superoxide dismutase, K^+, Na^+, K^+ : Na^+ of wheat cultivars both under saline and non-saline conditions.

Parameter	Genotype	Control (2 dS/m)		Saline (10 dS/m)	
		Si-	Si+	Si-	Si+
Soluble proteins (mg ml^{-1})	Auqab-2000	0.10^{de}	0.13^{a}	0.07^{f}	0.09^{e}
	SARC-5	0.12^{bc}	0.14^{a}	0.05g	0.11^{cd}
CAT (IU mg^{-1} of protein)	Auqab-2000	6.75^{a}	6.62^{a}	2.75^{d}	5.25^{b}
	SARC-5	4.17^{c}	4.21^{c}	2.72^{d}	4.14^{c}
POD (IU mg^{-1} of protein)	Auqab-2000	10.16^{d}	7.44^{e}	6.48^{f}	6.36g
	SARC-5	15.09^{a}	11.15^{b}	10.73^{c}	10.08^{d}
SOD (IU mg^{-1} of protein)	Auqab-2000	4.05^{b}	4.09^{bc}	2.04^{e}	3.73^{c}
	SARC-5	4.99^{a}	4.86^{a}	2.77^{d}	4.53^{ab}
K^+ (mg g^{-1} of dry weight)	Auqab-2000	10.74^{c}	15.43^{a}	6.25^{e}	9.39^{d}
	SARC-5	11.10^{c}	13.56^{b}	7.03^{e}	10.94^{c}
Na^+ (mg g^{-1} of dry weight)	Auqab-2000	5.88^{e}	3.90^{fg}	33.67^{a}	25.37^{c}
	SARC-5	5.36^{e}	3.41^{fg}	32.37^{d}	16.75^{b}
K^+ : Na^+	Auqab-2000	1.84^{b}	3.65^{a}	0.18^{d}	0.37^{cd}
	SARC-5	2.21^{b}	3.98^{a}	0.22^{d}	0.65^{c}
Si (mg g^{-1} of dry weight)	Auqab-2000	9.86^{c}	17.30^{b}	6.03^{d}	17.10^{b}
	SARC-5	11.04^{c}	23.96^{a}	9.86^{c}	22.75^{a}

$p \leq 0.05$ (the values are means of three replicates). Si- and Si+ represent 0 and 150 mg kg^{-1} of Si respectively.

Effect of Si application on total soluble proteins and antioxidants of wheat

Total soluble proteins

Soluble proteins, CAT, POD and SOD (Table 2) in flag leaves were significantly decreased in the presence of NaCl in both cultivars as against the control. The protein contents were increased significantly with the application of Ca-silicate not only under saline but also under non-saline conditions in both cultivars when compared with Si-deprived treatment. Si supplementation increased CAT, SOD concentration significantly under saline conditions in both the cultivars in comparison to no Si application. However, Si application remained ineffective to change CAT and SOD under non-saline conditions. It is antagonistic to note that Si supplementation reduced POD concentration when compared with plants where Si was not added. Comparing both the cultivars, under non-saline conditions Auqab-2000 was better than SARC-5, whereas under saline conditions, SARC-5 had more total soluble proteins and improved defense activities than Auqab-2000.

Effect of Si application on dry matter yield

Dry matter yield was significantly decreased in the presence of NaCl in both cultivars as against control (Table 1). The dry matter yield increased significantly with the application of Ca-silicate not only under saline but also under non-saline conditions in both cultivars when compared with Si-deprived treatment. Comparing both the cultivars, under non-saline conditions Auqab-2000 produced more than SARC-5, where as under saline conditions, SARC-5 had greater total dry weight than Auqab-2000.

Correlations

A significant ($p < 0.05$) positive regression co-efficient

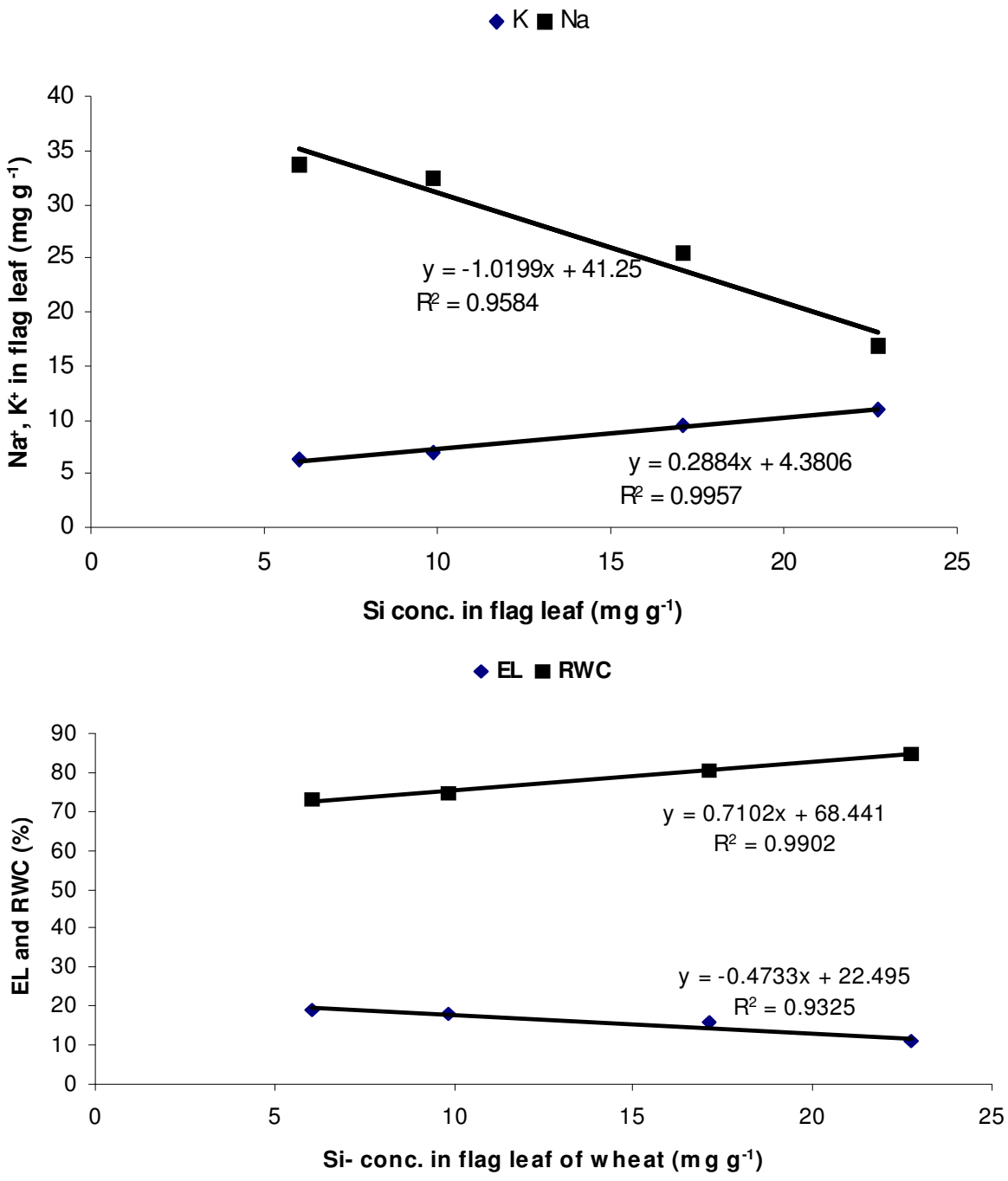

Figure 1. Schematic diagrams showing regression coefficient relationship between Si and N^+, K^+, SOD and CAT concentration, electrolyte leakage and relative water content leaves of wheat in saline culture.

relationship existed between Si and K^+, RWC, SOD (R^2 = 0.99, n = 4) and CAT (R^2 = 0.55, n = 4) in flag leaf under saline conditions. But, it was negative between Si and Na^+ (R^2 = -0.95, n = 4) and EL (R^2 = -0.93, n = 4) in wheat leaves (Figure 1a, b and c).

DISCUSSION

Excess or deficiency of any mineral nutrient is crucial for the reason that the plant growth depends on supply of inorganic nutrients (Marschner, 1995). Ionic imbalance

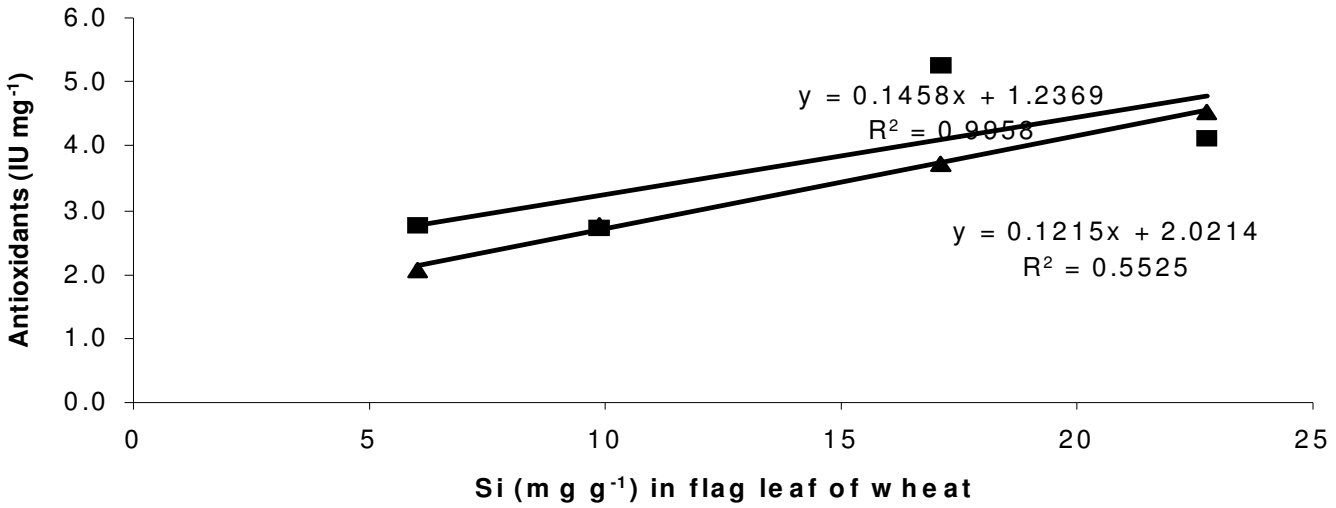

Figure 1. Contd.

occurs in the cell due to excessive accumulation of Na^+ which reduces the uptake of other essential mineral nutrients including K^+ (Lutts et al., 1999). Wheat plants also inevitably accumulate high concentration of Na^+ ions in their leaves under saline conditions (Gong et al., 2006). The current data also showed that the salt stress (EC = 10 dS m^{-1}) significantly ($p < 0.05$) increased Na^+ content in flag leaves of wheat plants and the uptake of Na^+ in Auqab-2000 (salt sensitive) was more obvious as compared to SARC-5 (salt tolerant). Any factor that suppresses the uptake and translocation of Na^+ in plant body eventually leads the plant to combat the salt stress. Si though, non-essential but its application in saline environment is beneficial (Raza et al., 2006). Wheat has also been designated as a Si-accumulator (Mayland et al., 1991). Therefore, the Si application (Si+) significantly increased the Si-content in flag leaves of wheat plants of both cvs under saline and non-saline conditions. Si uptake after its deposition within plant body significantly reduced the concentration of Na^+ in leaves of both cultivars under saline and non-saline conditions. Si reduces transportation of Na^+ to aerial parts of plants by reducing the activity of free Na^+ in solution by making a complex with Na^+ (Matoh et al., 1986; Ahmad et al., 1992), but also by decreasing the internal contents of Na^+ (Matoh et al., 1986). Si also inhibits Na^+ uptake and its onward translocation to shoots partly by the inhibitory effect of Si on the transpiration stream (Yeo et al., 1999) after its deposition in exodermis and endodermis of roots (Gong et al., 2003). Salt stress (Table 2) reduced K^+ concentration in both cultivars and Liang et al. (1999) also reported a significant reduction in K^+ ions in shoots of barley plants when exposed to saline stress. Decreased Na^+ concentration in plants shows lower Na^+ uptake and the ability of plants to combat the salinity stress that will strongly depend upon K^+ content. The addition of silicate @ 150 mg L^{-1} significantly ($p < 0.05$) increased the K^+ concentration than the plants grown without Si both under normal and saline conditions. This implied that K^+ transport was improved by Si application indicating that the effect of Si is supposed to alter the flux through K^+ ion transporters.

Liang et al. (1999) found that the salinity tolerance due to Si application is accredited by selective uptake and transport of K^+ and Na^+ by plants and the major proportion of Na^+ taken up by plant roots stays in the apoplast in some species. So, Si uptake is positively correlated with K^+ and negatively with Na^+ uptake. The current data showed that added NaCl into the solution culture (EC = 10 dS m^{-1}) significantly increased the electrolyte leakage in both cultivars by impairing the membrane stability possibly due to water stress (physiological drought) as suggested by (Tabaei-Aghdaei et al., 2000). The extent of electrolyte leakage was more in Auqab-2000 than SARC-5. Exogenous application of Si in saline growth medium reduces electrolyte by maintaining the integrity and functions of membrane, thus mitigating salt toxicity (Liang et al., 2003). The present study showed that addition of Ca-silicate into the solution culture decreased electrolyte leakage under salt stress showing a negative correlation between Si content and electrolyte leakage. This ameliorative effect of Si may be due to its hydrophilic nature by maintaining plant water status and by protecting the plants from physiological drought (Murillo-Amador, 2007). Salt stress imposes an oxidative stress in addition to the ion toxicity and osmotic stress (Guetadahan et al., 1998) that can stimulate the production ROS species such as superoxide, hydrogen peroxide, hydroxyl

radical and singlet oxygen (Lee et al., 2001) and attack nucleic acids, proteins and lipids (Menezes-Benavente et al., 2004). The activity of antioxidants (CAT, SOD and POD) was suppressed by salt stress (Na^+) leading the wheat plants of both cultivars towards weak defense system due to increased production of ROS. Salinity not only suppressed the antioxidant enzymes but it suppressed the overall protein production.

The salinity damage was associated with the accumulation of Na^+ in leaf tissues followed by reduction of enzymatic processes and protein synthesis (Tester and Davenport, 2003). Si moderately offsets the negative effects of salt stress by enhancing SOD and CAT activeties and soluble proteins in tomato (Al-aghabary et al., 2004), cucumber (Zhu et al., 2004) and barley (Liang et al., 1996). Si application at 150 mg L^{-1} in wheat excited the scavenging system and promoted the production of SOD and CAT and Solule proteins in both cultivars indicating a positive relationship between Si and SOD and CAT under saline conditions. These results support the idea of Moussa (2006) and Gong et al. (2005). It is interesting to note that in the present study, added Si significantly increased the SOD and CAT activity, but decreased the POD activity. It indicated that the oxidative damage due to salt stress was ameliorated by Si addition due to the remarkable increase of SOD and CAT and decrease of H_2O_2 content (Liang, 1996). Data show that chlorophyll contents and their ratios were significantly reduced in the presence of NaCl in comparison to control as reported by Chen et al. (1991). Si treatments increased the chlorophyll contents and its ratios under saline conditions. Among cultivars, SARC-5 contained Chl a higher in comparison to Auqab-2000 under control conditions, but under saline environments both were observed at same status. The data revealed that applied Si into nutrient solutions resulted in an obvious increase in Chl a content as compared to Chl b that enhanced the photosynthetic efficiency in salt-stressed plants by stimulating the activity of photosynthesis. Current findings supported the results of Al-aghabary et al. (2004) and Moussa (2006) in tomato and maize, respectively showing an improvement in the photosynthetic efficiency due to Si. It can be suggested that addition of Si improved plant defense system to detoxify ROS induced under salt stress, which in turn helped to increase chlorophyll and enhanced the photochemical efficiency of PS-II. It is also confirmed that scavenging system is primary defense line against oxidative stress induced by salt stress.

The salinity damage is also associated with increased transpiration resulting into lower water content in plant leaves (Munns, 2005). In the present study, when salinity was imposed on wheat, it lowered the relative water content (RWC) in both cultivars. Si application at 150 mg L^{-1} increased the RWC to significant extent and a positive correlation was found between Si and RWC in leaf under saline conditions. Results are in conformity with those of Gong et al. (2003). The higher RWC in salt-stressed plants in the presence of Si was attributed to the reduction in excessive loss of water by transpiration. It was suggested that Si was deposited in the epidermal cells of aerial parts of plants, leaves and stems that decreased the water loss through the cuticle and the higher water content maintained by Si diluted the salts in plant body and consequently reduced deleterious effects of salinity on plant growth (Romero et al., 2006). Hence in the current study, Si amendment increased the dry matter yield both under saline and non-saline conditions in both cultivars. The current experiment suggested that SARC-5 performed better than Auqab-2000 under saline conditions and proved to be salt tolerant. These results supported the findings of Saqib et al. (2004) indicating some Na^+ exclusion mechanism in this cultivar. Auqab-2000 is more sensitive to salinity than SARC-5 (Tahir et al., 2006). It can be concluded from current research that Si application improves the growth, physiological and metabolic performance in wheat under salinity stress. Salinity tolerance in Si-applied wheat is attributed to reduced uptake of Na^+ and enhanced uptake of K^+ contributing to more balanced nutrition. Si application maintains higher water status, photosynthetic activity and better plant defense system and membrane permeability/stability. However, there is a dire need to conduct further studies to verify results of water culture in soil medium.

ACKNOWLEDGEMENTS

We are extremely thankful to the Department of Crop Physiology, University of Agriculture, Faisalabad for facilitating us by making an arrangement for successful execution of lab work. We express our gratitude to Dr. Muhammad Shahid, Department of Biochemistry, University of Agriculture, Faisalabad, Pakistan for his efforts in the analysis of antioxidants.

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