***Pak. J. Bot.*, 49(3): 823-835, 2017.**

SILICON IMPROVED GROWTH RATE BY ALTERING BIOCHEMICAL TRAITS OF WHEAT UNDER SALINE CONDITIONS

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Abstract

Silicon (Si) is a constructive element for plant growth. Its application plays a multiple role to increase plant growth through influencing physiological and metabolic processes in higher plants under both biotic and abiotic stresses. A hydroponic study was conducted to investigate the interactive effect of salinity and silicon (Si) on plant biochemical attributes at two different growth stages by growing two wheat varieties (Faisalabad-2008 as salt tolerant & Millat-2011 as salt sensitive). The two salinity (100 and 200 mM NaCl) and two silicon (1 mM and 2 mM H_2SiO_3) levels were applied using ½ strength Hoagland's nutrient solution in a wire house for 30 days. It was found that Si significantly increased the salt tolerance of both wheat varieties by improving the plant biochemical traits. Faisalabad-2008 performed better than Millat-2011at different growth stages, i.e., (tillering and stem elongation).

Key words: Wheat, Salinity, Silicon, Antioxidant enzymes.

Introduction

Salinity is the major yield constraining problem in many parts of the world (Haq *et al*., 2010; Fahad *et al*., 2015), especially in regions having arid to semiarid climate (Schleiff, 2008). It affects crop production through a number of ways which include osmotic stress, nutrient imbalance and specific ion toxicity in the plant tissues (Munns, 2005; Abbasi *et al*., 2015a; Wu *et al*., 2014). Reactive oxygen species (ROS) produced during various stresses are the major cause of cell degradation (Li *et al*., 2010) and serious threat to plants because they deteriorate the plant defense system, reduce growth, impair gene expression and disturb stress signaling mechanisms (Gill & Tuteja, 2010).

Wheat (*Triticum aestivum* L.) is an important food crop because it is nutritionally very rich. It is a moderately salt tolerant crop (Mass & Hoffman, 1977) which is badly damaged due to salinity stress (Zhu, 2003). So it is vital to sustain wheat production by using the available resources efficiently to ensure food security.

Mineral nutrition has been proved effective to minimize the adverse effects of salinity stress on plants (Munns *et al.,* 2006). Among mineral elements, silicon has played an important role in various studies to ameliorate the salinity stress in various crops. It is a beneficial element in plant growth, however, its influence on biological processes is not clear (Wang & Han, 2007). Silicon is the predominant component comprising 25% of earth crust (Sommer *et al.*, 2006). It has a constructive impact on plant growth like wheat crop (Tahir *et al*., 2012; Tuna *et al*., 2008). It is evident from various studies that plant growth can be increased by Si application under normal or stressful environments (Hattori *et al.,* 2008; Ashraf *et al*., 2009: Keller *et al*., 2015). Many scientists have suggested that Si may reduce the adverse effects of salt stress by influencing physiological and metabolic processes in higher plants (Liang *et al.,* 2007; Rizwan *et al*., 2015). The negative effects of NaCl stress is moderately compensated with Si application by improving the activities of antioxidant enzymes like CAT, SOD and non-enzymatic soluble proteins (Al-aghabary *et al*, 2004). Liang *et al*. (1996) reported that enzyme activities were considerably increased in barley plants with addition of silicon (Si) under saline stress. The higher activities of APX, SOD, GPX, GR and DHAR were observed in plant leaves treated with silicon in saline conditions which support plants to cope with oxidative damage under salinity stress (Zhu *et al*., 2004). Compatible solutes are synthesized in the cytoplasm and toxic ions are accumulated in the vacuoles (Bartels & Sunkar, 2005). Under stress conditions, plants tends to produce and collect compatible solutes like proline and other low molecular weight organic solutes (Ashraf & Foolad, 2007). The low proline concentration in non-saline conditions and high concentration in stress conditions is reported as common mechanisms of salt tolerance (Ketchum *et al.,* 1991).

Before the 20th century, the effect of silicon on growth of plant and stress tolerance remained ignored. Most of the plant physiologists overlooked its beneficial aspects on plant growth due to the following reasons: (1) it is a non-reactive element in soil-plant biological system and (2) lack of deficiency or toxicity symptoms on plant growth being its abundance in the nature and as a plant major inorganic constituent (Richmond & Sussman, 2003). Because of intensive cropping system and constant use of chemical fertilizers, soil becomes deficient in plant available Si as a nutrient. Now the Si has been recognized as production limiting factor in many soils, especially in highly weathered ones (Datnoff, 2004).

Based on the existing literature, it gives the impression that silicon (Si) in the symplast may be involved actively in biochemical alterations by modifying the antioxidant defense system of plants and synthesis of osmolytes production, hence increasing salt tolerance in plants. Keeping in view the above narrated considerations and importance of wheat crop, the present study was planned to investigate the ability of silicon to improve biochemical traits in two contrasting wheat varieties under salt stress conditions at two different growth stages.

Materials and Methods

The study was conducted in a wire house at the Institute of Soil and Environmental Sciences, University of Agriculture, Faisalabad. Healthy and sterilized seeds of two wheat varieties (Faisalabad-2008 as salt tolerant and Millat-2011 as salt sensitive) were grown in ½ strength Hoagland's nutrient solution (Hoagland & Arnon, 1950). Two levels of silicon (1 and 2 mM H_2SiO_3) and two levels of salinity (100 and 200 mM NaCl) with their respective control were applied with six replications according to a completely randomized design (CRD) with factorial arrangement. Two harvests were taken during the experiment. First harvest was taken at the tillering growth stage (15 days after salt stress) and second at the stem elongation / jointing stage (after 30 days of salt stress).

Relative growth rate was measured as unit increase in shoot dry weight per gram per day. The shoot dry weight for each variety was taken at 15 days and 30 days after final salt concentrations (100 and 200 mM NaCl) in 3 replicates.

$$\text{Relative growth rate (RGR)} = \frac{(\text{SDW2 - SDW1})}{(\text{T2 - T1})}$$

where SDW is the shoot dry weight and T is time in days.

The plant samples were digested following a conventional process (Keller *et al.,* 2005). Approximately 0.2 g (ground) plant samples were put in polytetrafluoroethylene tubes (Teflon tubes), 10 ml of di-acid mixture, nitric acid and perchloric acid (HNO_3:$HClO_4$ ratio of 2:1) were added to dissolve the organic matrix and incubated over- night at room temperature. Tubes were kept on a digestion plate and heated up to 115°C until vapors appeared, and the process continued to heat for 30 minutes. Then digestion tubes were removed and allowed to cool. The resulting material was filtered and diluted 25 times and the filtrate was used for further ionic analysis. The concentrations of sodium (Na^+) and potassium (K^+) were determined using a flame photometer (Sherwood-410). Silicon in plant material was determined by the colorimetric molybdenum blue method (Elliot & Snyder, 1991). To 1 ml of supernatant filtrate liquid, 25 ml of 20% acetic acid, 10 ml of ammonium moblybdate (54 g/L) solution were added in a 50 ml of polypropylene volumetric flask. After 5 minutes, 5 ml of 20% tartaric acid and 1 ml of reducing solution were added in a flask and the volume was made with 20% citric acid. After half hour, the absorbance was measured at 650 nm with a spectrophotometer (Shimadzu, Japan). The reducing agent was prepared by dissolving 1 g Na_2SO_3, 0.5 g 1 amino-2-naphthol-4-sulfonic acid and 30 g $NaHSO_3$ in 200 ml water.

Total protein was estimated using bovine serum albumin (Bradford, 1976). The reaction mixture contained 80 µl distilled water, 20 µl enzyme extract and 5 ml Coomassie solution. The test tube containing reaction mixture was shaken on a vortex and absorbance was noted at 595 nm on a spectrophotometer.

For determination of enzymes, the fully expanded fresh wheat leaves (0.5 g) were harvested and ground in a pestle and mortar by adding 10 mL of 50 mM ice cold phosphate buffer having pH 7.8. Then the centrifugation of homogenized leaf samples was done at 15000 x *g* for a time of 20 min at 4 °C. The activity of SOD was measured by its capability to inhibit the photo-reduction of nitroblue tetrazolium (NBT) (Giannopolitis & Ries, 1977) while the activity of catalase (CAT) and peroxidase (POD) was determined in the enzyme extract following the method defined by Chance & Maehly (1955).

Proline contents in the wheat leaf samples were measured following the method of Bates *et al*. (1973). Approximately, 0.5 g leaf sample was ground in the presence of 10 mL of sulfo-salicylic acid (3%). Two (2) mL of the filtrate were put in a test tube and allowed to react with 2 mL acid ninhydrin solution (1.25 g ninhydrin + 30 mL glacial acetic acid + 20 mL of 6 M orthophosphoric acid) and then 2 mL glacial acetic acid were also added. The reaction mixture was heated at 100°C and after 1 hour the reaction was ended using an ice bath. Then the extraction of the mixture was done using 10 mL toluene which turned to form a chromophore. To separate the aqueous phase from the toluene containing chromophore, aeration was given for 2 min in the reaction mixture. The extracted samples (colored phase) were kept for 2-3 min. and absorbance was read at 520 nm on a spectrophotometer.

Statistical analysis: Data recorded for various observations were statistically analyzed by adopting analysis of variance technique based on completely randomized design with factorial arrangement, using the computer software; STATISTIX 8.1. The means of genotypes and treatments were compared by Turkey's HSD (honest significant difference) test, at 5% probability.

Results

Relative growth rate (g g^{-1} day^{-1}): The data (Fig. 1) regarding relative growth rate (RGR) of both wheat varieties (Faisalabad-2008 as salt tolerant and Millat-2011 as salt sensitive) indicated that salinity reduced the relative growth rate of both the varieties significantly ($p \leq 0.05$) at both salinity levels. A considerable increase in relative growth of both the varieties was observed with the addition of Si. The maximum relative growth rate was observed for Faisalabad-2008 at Si level 1 mM under control conditions. The maximum RGR (0.117) was exhibited by Faisalabad-2008 in comparison with RGR (0.044) of Millat-2011 plants treated with Si @ 2 mM under both salinity levels 200 mM NaCl). There was a significant difference in RGR between both varieties at 200 mM salinity level. Faisalabad-2008 performed best at high salt stress while at low salinity level Millat-2011 showed relatively better RGR.

Silicon (Si) concentration in shoot: According to statistical analysis of data (Fig. 2), main and interactive effects between the wheat varieties and treatments were found significant ($p \leq 0.05$). Silicon concentration increased significantly at both Si levels (1 and 2 mM) both under normal and saline conditions. The differences in Si concentration were found statistically non-significant between both Si levels. Silicon (Si) uptake into the plants at both salinity levels (100 and 200 mM) increased as compared to non-saline conditions. The maximum Si concentration (8.16 mg g^{-1}) was detected in shoot of Faisalabad-2008 at the stem elongation stage in the plants treated with Si @ 2 mM, while the minimum Si concentration (0.67 mg g^{-1}) was determined in the shoots of Millat-2011 at the tillering stage where no Si was added under salinity stress 200 mM NaCl. The stem elongation growth stage (SEGS) showed significantly higher concentration of Si in shoot than tillering growth stage (TGS) in both wheat varieties.

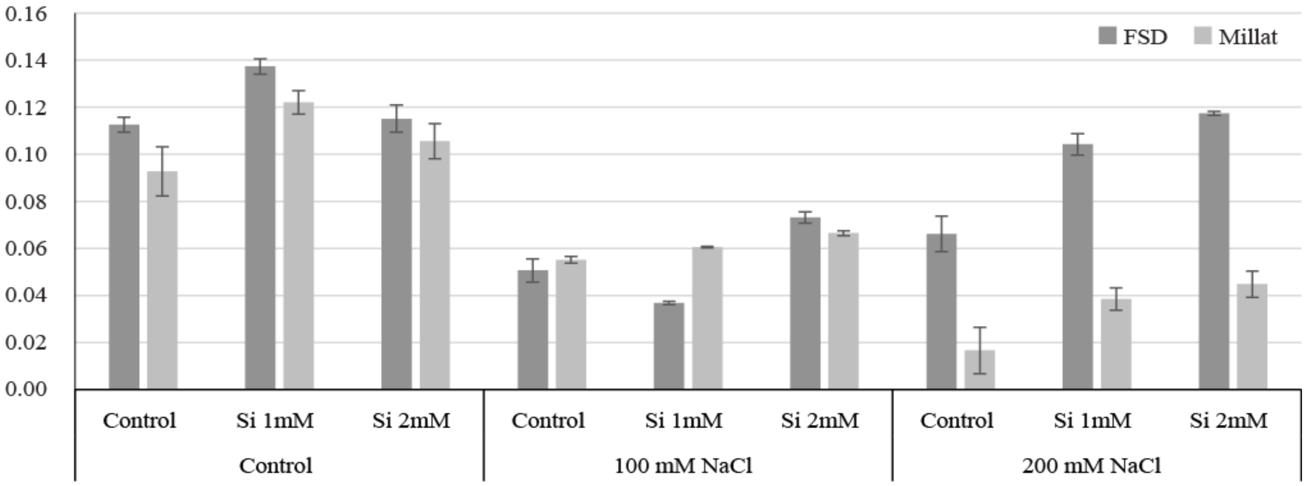

Fig. 1. Effect of Si (H_2SiO_3) on relative growth rate (RGR) of wheat under saline condition; the values are means of three replications ± standard error.

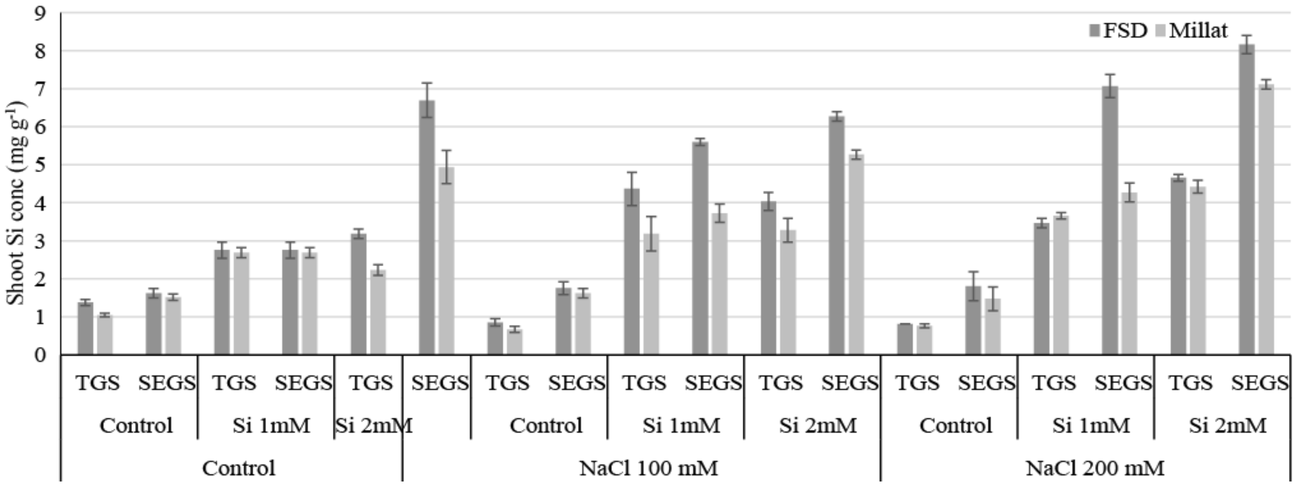

Fig. 2. Effect of Si (H_2SiO_3) on Silicon (Si) concentration in shoot of wheat at tillering (TGS) and stem elongation growth stage (SEGS) under saline condition; the values are means of three replications ± standard error.

Silicon (Si) concentration in root: Silicon concentration in root (Fig. 3) increased significantly at both Si levels (1 and 2 mM) under saline and non-saline conditions. The differences in Si concentration were found statistically non-significant between both Si levels. The higher Si concentration was observed in the roots of Faisalabad-2008 as compared to that in cv. Millat-2011. The stem elongation growth stage (SEGS) showed significantly higher concentration of Si in root than at the tillering growth stage (TGS) in both wheat varieties.

The maximum Si concentration (6.41 mg g^{-1}) was detected in the roots of cv. Faisalabad-2008 at the stem elongation stage in the plants treated with Si @ 2 mM, under non-saline conditions while the minimum Si concentration (1.29 mg g^{-1}) was determined in the roots of Millat-2011 at the tillering stage where no Si was added under salinity stress 100 mM NaCl. The results also indicated that root silicon (Si) concentration increased under salt stress conditions as compared to control (non-saline condition).

Sodium (Na^+) concentration in shoot: Data (Fig. 4) showed significant differences among treatments and wheat varieties as well as their interactions on the sodium (Na^+) concentration in the shoot of wheat plants. Sodium (Na^+) concentration in the shoot of both wheat varieties increased with addition of NaCl in the nutrient solution. Addition of silicon (Si) to the nutrient solution considerably ($p\leq0.05$**)** reduced Na^+ concentration in the shoot at both levels of salt stress. Reduction in sodium (Na^+) concentration at Si level 2 mM was more as compared to Si level 1 mM in both selected varieties.

Relatively higher concentration of Na^+ was determined in the leaves of Millat-2011 while Faisalabd-2008 maintained less concentration of Na^+ in its leaves. The maximum Na^+ concentration (52.05 mg g^{-1}) was observed in the shoot of Millat-2011 at the stem elongation stage in the plants treated with no Si in comparison with Na^+ concentration (43.43 mg g^{-1}) in the shoots of Faisalabad-2008 under salinity stress 200 mM NaCl, while the minimum Na^+ concentration (2.76 mg g^{-1}) was determined in the shoots of Faisalabad-2008 at the tillering stage in the plants treated with Si @ 2 mM in comparison with Na^+ concentration (8.02 mg g^{-1}) in the shoots of Millat-2011 under non-saline conditions.

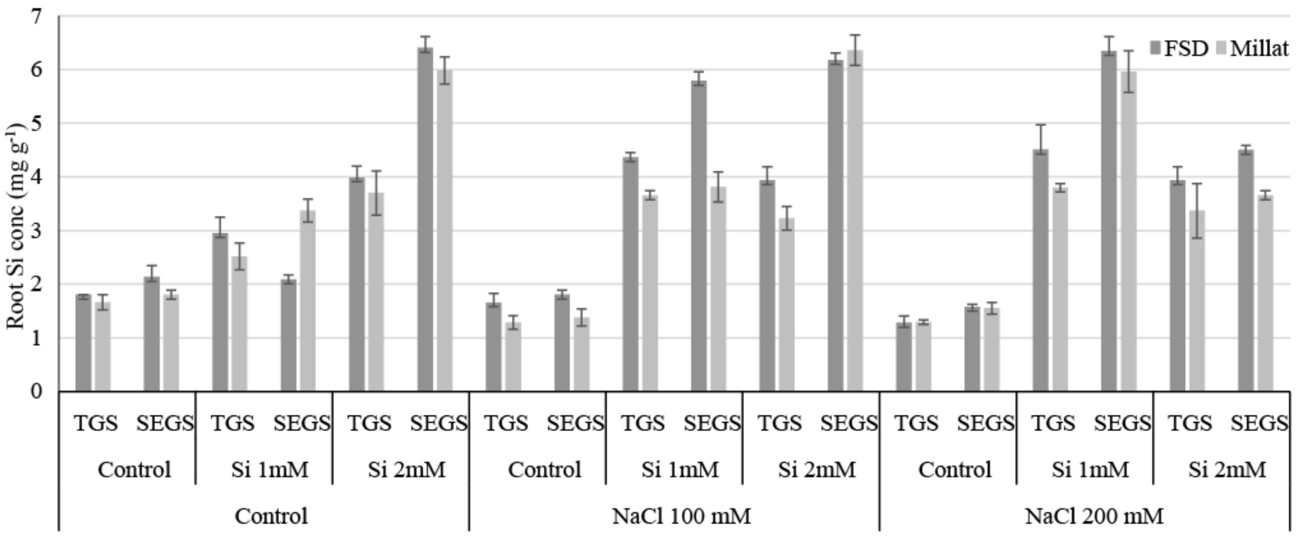

Fig. 3. Effect of Si (H_2SiO_3) on Silicon (Si) concentration in root of wheat at tillering (TGS) and stem elongation growth stage (SEGS) under saline condition; the values are means of three replications ± standard error.

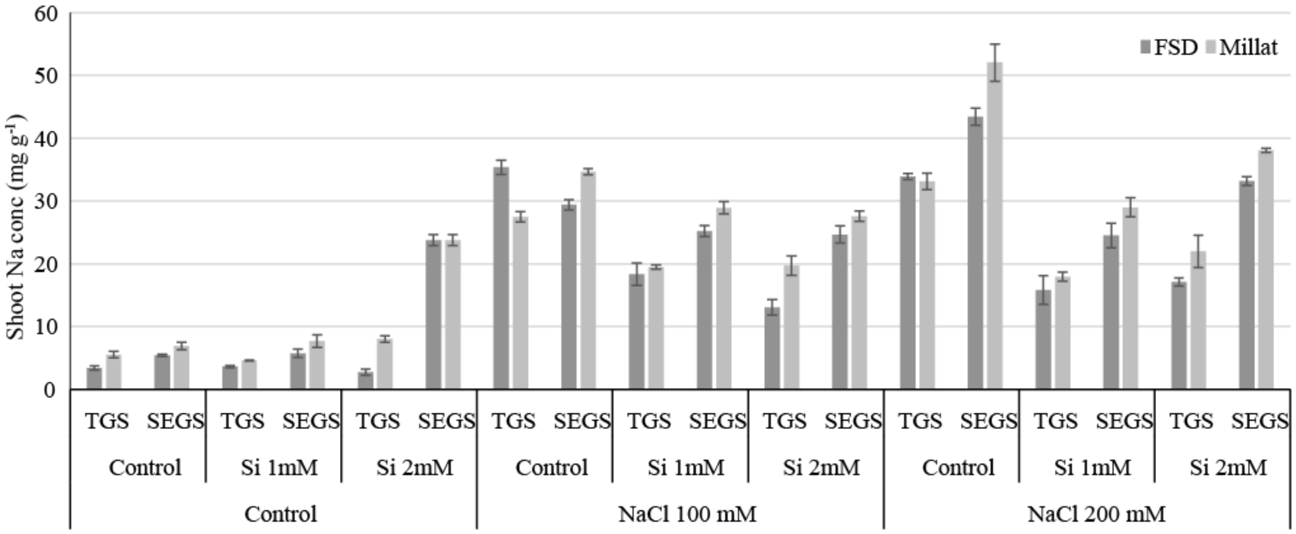

Fig. 4. Effect of Si (H_2SiO_3) on Sodium (Na^+) concentration in shoot of wheat at tillering (TGS) and stem elongation growth stage (SEGS) under saline condition; the values are means of three replications ± standard error.

Sodium (Na^+) concentration in root: Data (Fig. 5) regarding Na^+ concentrations in the root showed that relatively higher Na^+ concentration was determined in the roots of Faisalabad-2008 at both salinity levels indicating its ability to reduce Na^+ translocation at root level in response to Si addition. Comparing the growth stages, higher concentration of Na^+ was found at the stem elongation stage. However, addition of silicon to the nutrient solution considerably ($p \leq 0.05$) reduced Na^+ concentration in the root at both levels of salt stress. The maximum Na^+ concentration (53.96 mg g^{-1}) was observed in the root of Faisalabad-2008 at the stem elongation stage in the plants not treated with Si in comparison with Na^+ concentration (48.35 mg g^{-1}) in the roots of Millat-2011under salinity stress 200 mM NaCl, while the minimum Na^+ concentration (6.25 mg g^{-1}) was determined in the shoots of Millat-2011, at the stem elongation stage in the plants treated with Si @ 1 mM in comparison with Na^+ concentration (14.82 mg g^{-1}) in the shoots of Faisalabad-2008 under non-saline conditions.

Potassium (K^+) concentration in shoot: According to statistical analysis of data (Fig. 6) there were significant ($p \leq 0.05$) differences among the treatments and wheat varieties as well as their interactions on the potassium (K^+) concentration in the shoots of both wheat varieties. Potassium (K^+) concentration in the shoots of both wheat varieties decreased with addition of NaCl to the nutrient solution. However, the higher K^+ concentrations were maintained by Faisalabd-2008 as compared to Millat-2011 at both salinity levels. Addition of silicon (Si) to the nutrient solution considerably enhanced K^+ concentration in the shoot at both levels of salt stress. Comparing the growth stages, relatively higher concentration of shoot K^+ was found at the stem elongation stage at non-saline conditions but significant difference in K concentration was observed between both growth stages. The highest K^+ concentration (26.87 mg g^{-1}) was detected in the shoots of Faisalabad-2008 at the stem elongation stage in the plants treated with Si @ 2 mM in comparison with K^+ concentration (21.60 mg g^{-1}) in the shoots of Millat-2011 under non-saline conditions, while the lowest K^+ concentration (7.82 mg g^{-1}) was determined in the shoots of Millat-2011, at the tillering stage in the plants without Si addition in comparison with K^+ concentration (10.26 mg g^{-1}) in the shoots of Faisalabad-2008 under salinity stress (100 mM NaCl).

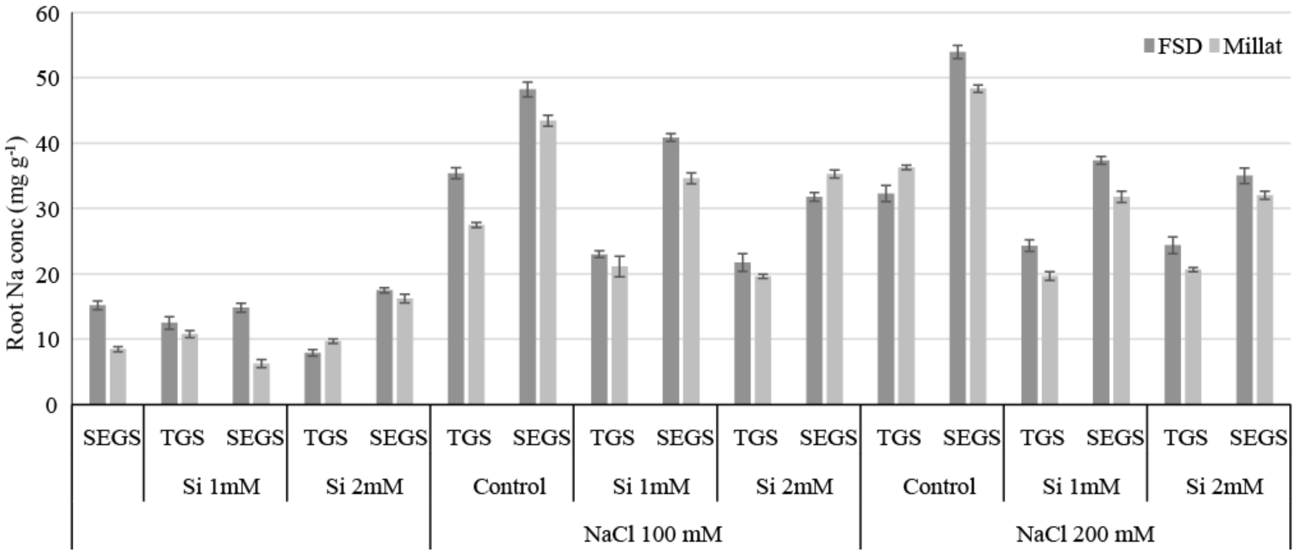

Fig. 5. Effect of Si (H_2SiO_3) on sodium (Na^+) concentration in root of wheat at tillering (TGS) and stem elongation growth stage (SEGS) under saline condition; the values are means of three replications ± standard error.

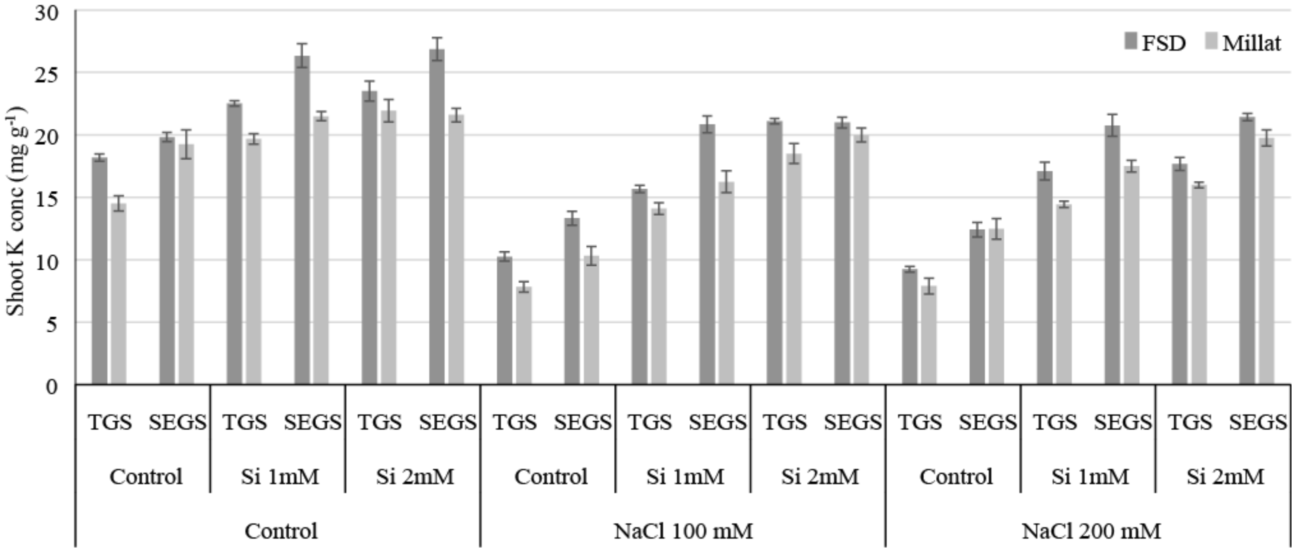

Fig. 6. Effect of Si (H_2SiO_3) on potassium (K^+) concentration in shoot of wheat at tillering (TGS) and stem elongation growth stage (SEGS) under saline condition; the values are means of three replications ± standard error.

Potassium (K^+) concentration in root: The salinity significantly ($p \leq 0.05$) decreased the K^+ concentration in the roots of both varieties (Fig. 7). The addition of Si increased the K^+ concentration significantly under both saline and non-saline conditions. Comparatively higher K^+ concentration was determined in the roots of Faisalabad-2008 than that in cv. Millat-2011, however, the differences between both Si levels were found non-significant. The more K^+ concentration was recorded at the stem elongation stage. The maximum K^+ concentration (21.84 mg g^{-1}) was found in the roots of Faisalabad-2008 at the stem elongation stage in the plants treated with Si @ 2 mM in comparison with-K^+ concentration (19.16 mg g^{-1}) in the roots of Millat-2011 under non-saline conditions, while the lowest K^+ concentration (4.87 mg g^{-1}) was determined in the roots of Millat-2011, at the tillering stage in the plants without Si addition in comparison with K^+ concentration (7.96 mg g^{-1}) in the roots of Faisalabad-2008 under salinity stress (200 mM NaCl).

K^+: Na^+ ratio in shoot: Data of $K^{+:}$ Na^+ ratio in the shoot indicated that salinity badly reduced K^+: Na^+ ratio in the leaves of both wheat varieties at both salinity levels when plants were exposed to salt stress (Fig. 8). The reduction in K^+: Na^+ ratio caused a suppression in uptake of potassium by the wheat plants and increased the Na^+ uptake which ultimately reduced the plant growth. Addition of Si increased the K^+: Na^+ ratio both under normal and saline conditions. The statistical analysis of data showed a significant interactive effect among salinity, silicon, varieties and growth periods. Wheat genotype Faisalabad-2008 performed better as it retained high K^+: Na^+ ratio in their leaves in comparison to Millat-2011variety. The higher K^+: Na^+ ratio was observed at tillering stage under both saline and non-saline conditions. Faisalabad-2008 showed the maximum K^+: Na^+ ratio (9.17) at the tillering stage in the plants treated with Si @ 2 mM in comparison with Millat-2011 (2.77) under non-saline conditions while the lowest value (0.24) was found in the leaves of Millat-2011 plants not treated with Si under saline conditions (200 mM NaCl).

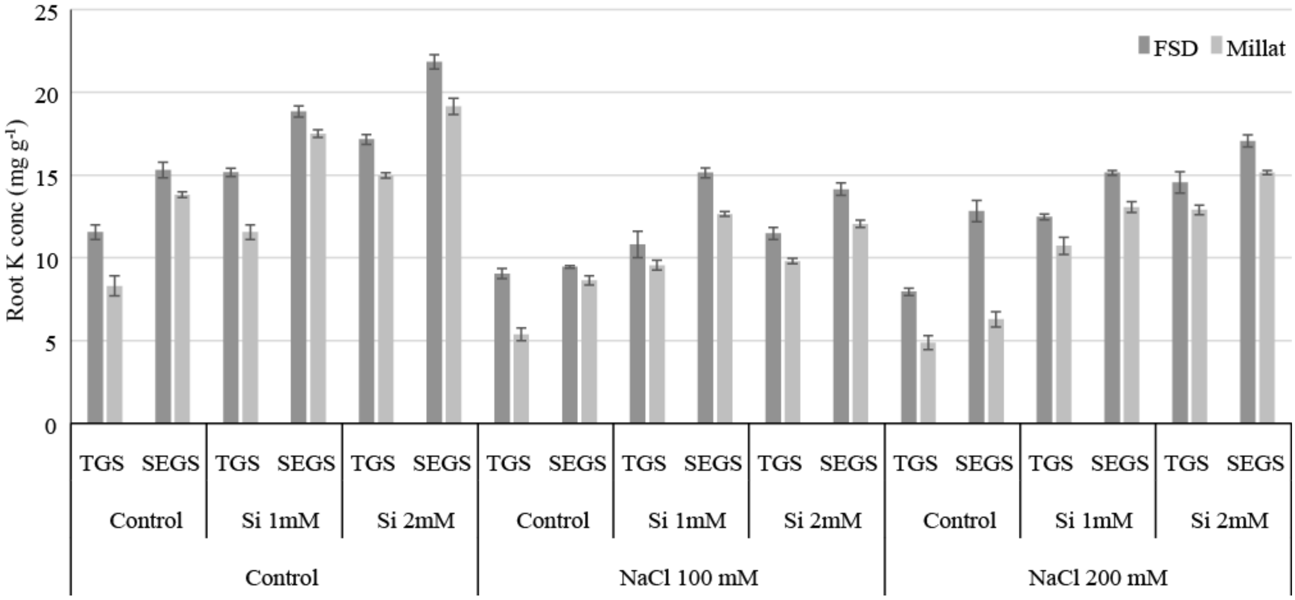

Fig. 7. Effect of Si (H_2SiO_3) on potassium (K^+) concentration in root of wheat at tillering (TGS) and stem elongation growth stage (SEGS) under saline condition; the values are means of three replications ± standard error.

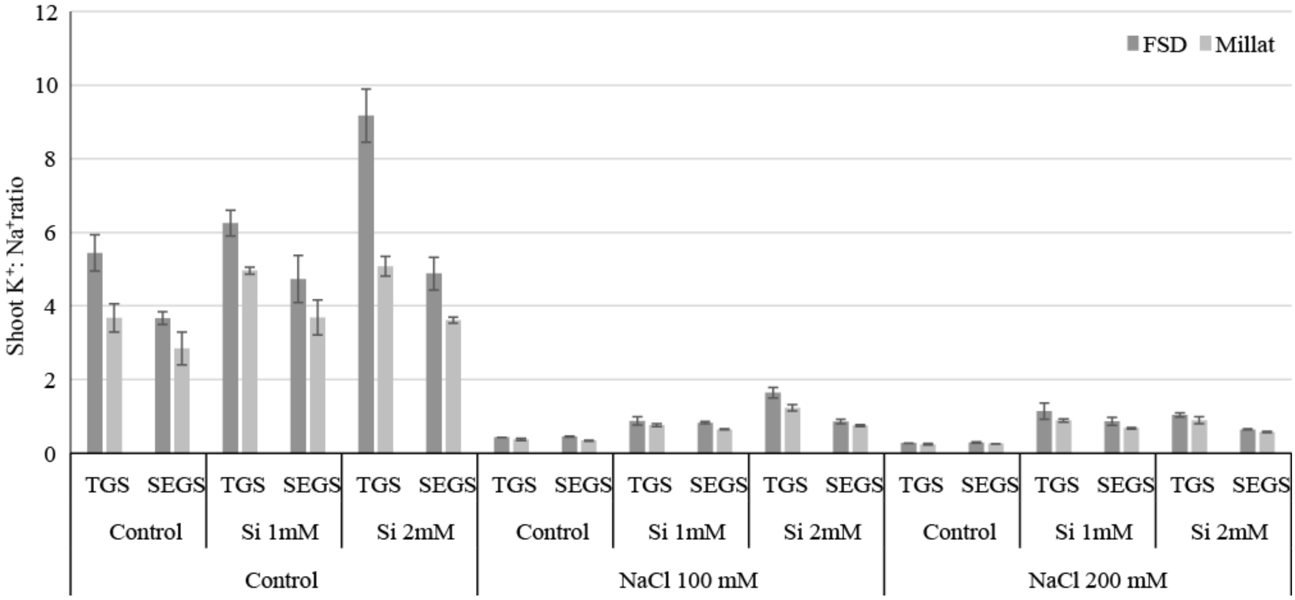

Fig. 8. Effect of Si (H_2SiO_3) on Shoot K^+: Na^+ ratio of wheat at tillering (TGS) and stem elongation growth stage (SEGS) under saline condition; the values are means of three replications ± standard error

Total protein: Data of total protein revealed that salinity considerably ($p \leq 0.05$) reduced the total proteins in both wheat varieties at both salinity levels (100 and 200 mM NaCl stress) as compared to control (Fig. 9). Millat-2011 showed low values of total protein in comparison with Faisalabad-2008. Relatively higher quantity of total proteins was observed at stem elongation growth stage both at normal and saline conditions. Addition of silicon (Si) remarkably ($p \leq 0.05$) enhanced the total proteins at both salinity levels as well as in normal conditions. Relatively higher concentration of total proteins were found at salinity level 200 mM NaCl where Si was used @ 2 mM. Comparing the varieties, Faisalab-2008 depicted significantly higher values of total proteins as compared to Millat-2011. Faisalabad-2008 showed the maximum protein contents (0.199) at the stem elongation stage in the plants treated with Si @ 1 mM in comparison with Millat-2011 (0.184) under non-saline conditions while the lowest value of protein contents (0.073) was found in the shoot of Millat-2011 plants without addition of Si at the tillering stage under salt stress (100 mM NaCl).

Superoxide dismutase (SOD): Salinity adversely affected the activity of superoxide dismutase (SOD) at both stress levels as compare to controlled condition (Fig. 10). Apparently there was no variation between both salt stress levels with respect to SOD activity. Addition of silicon (Si) ameliorated the negative effect of salinity by increasing SOD activity in the leaves of salt stressed plants where as the activity of SOD significantly ($p \leq 0.05$**)** reduced under non-saline condition. Minimum value of SOD was observed under normal condition where Si was applied @ 2mM. Comparing the wheat varieties, Faisalabad-2008 showed significantly enhanced activity of SOD. Relatively higher activity of SOD was observed at the stem elongation growth stage as compared to that at the tillering stage both under normal and saline conditions. The maximum SOD activity (6.35 unit mg^{-1} protein) was found in the shoot of Faisalabad-2008 at the tillering stage in the plants treated with Si @ 2 mM in comparison with SOD activity (5.00 unit mg^{-1} protein) in the shoot of Millat-2011 under saline conditions (200 mM NaCl), while the lowest SOD activity (1.67 unit mg^{-1} protein) was determined in the shoot of Millat-2011 at the stem elongation stage in the plants treated with Si @ 2 mM under non-saline conditions. A positive correlation (R=0.66, n=36) was found between shoot Si concentration and activity of SOD under saline conditions (Fig. 15).

Catalase (CAT): The activity of catalase (CAT) in the leaves of both varieties decreased significantly ($p \leq 0.05$) with the application of salt stress in the nutrient solution in comparison with normal condition (Fig. 11). There was no significant variation in the concentration of CAT under stress condition. A significant increase in the activity of CAT was observed in response to Si application under both stress conditions. Conversely, the activity of CAT was prominently reduced under normal conditions in response to applied Si. The maximum increase in CAT concentration was found in Faisalabad-2008 as compared to that in Millat-2011. Minor variations in CAT concentration were observed between both growth stages. The highest CAT activity (7.80 unit mg^{-1} protein) was measured in the shoot of Faisalabad-2008 at the stem elongation stage treated with Si @ 2 mM under saline conditions (100 mM NaCl), while the minimum CAT activity (1.65 unit mg^{-1} protein) was detected in the shoot of Millat-2011treated with Si @ 1 mM at the tillering stage under non-saline conditions. A positive correlation (R=0.22, n=36) was found between shoot Si concentration and activity of catalase (CAT) under saline conditions (Fig. 15).

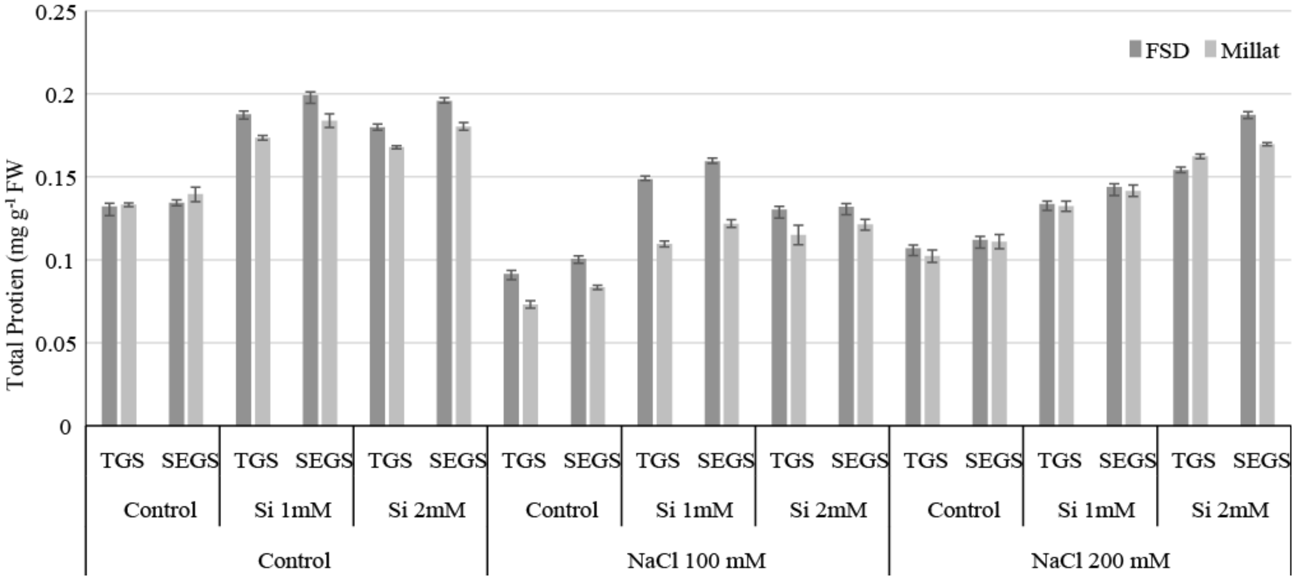

Fig. 9. Effect of Si (H_2SiO_3) on total proteins in wheat leaves at tillering (TGS) and stem elongation growth stage (SEGS) under saline condition; the values are means of three replications ± standard error.

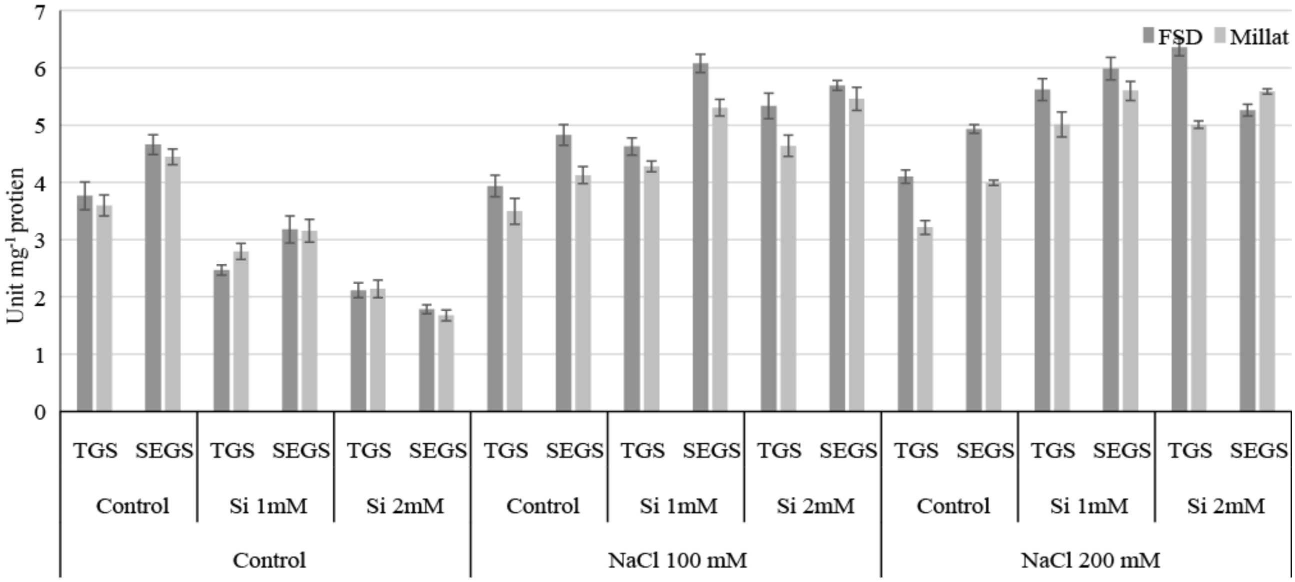

Fig. 10. Effect of Si (H_2SiO_3) on SOD activity in wheat leaves at tillering (TGS) and stem elongation growth stage (SEGS) under saline condition; the values are means of three replications ± standard error.

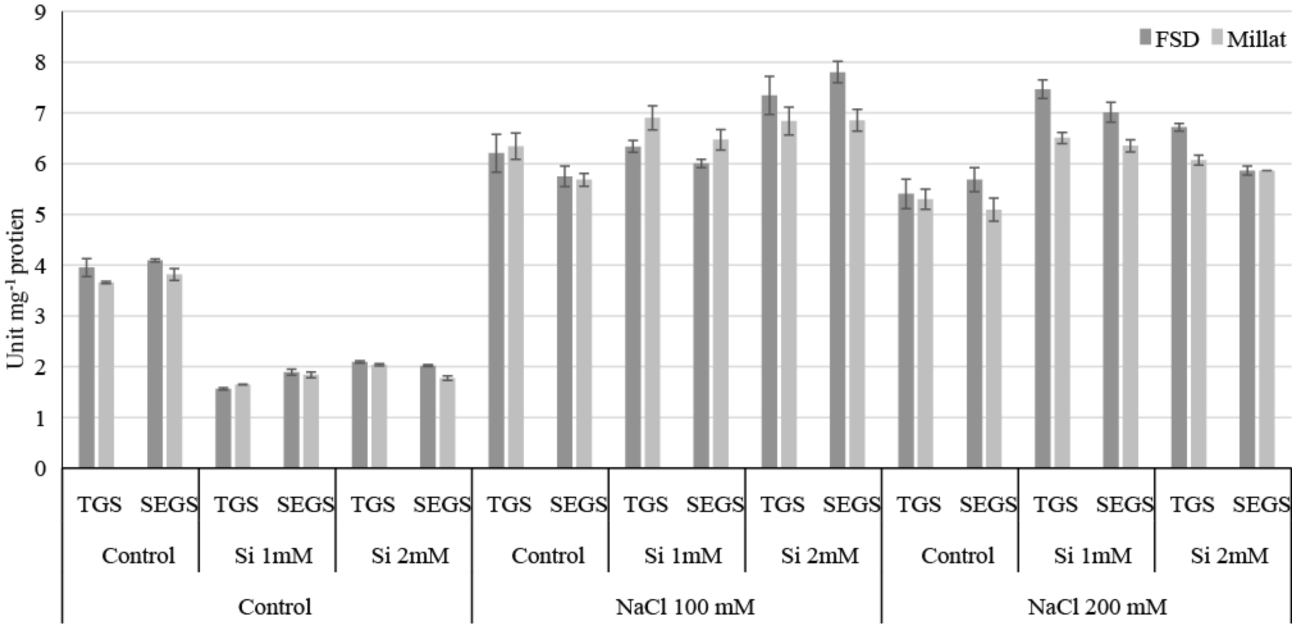

Fig. 11. Effect of Si (H_2SiO_3) on CAT activity in wheat leaves at tillering (TGS) and stem elongation growth stage (SEGS) under saline condition; the values are means of three replications ± standard error.

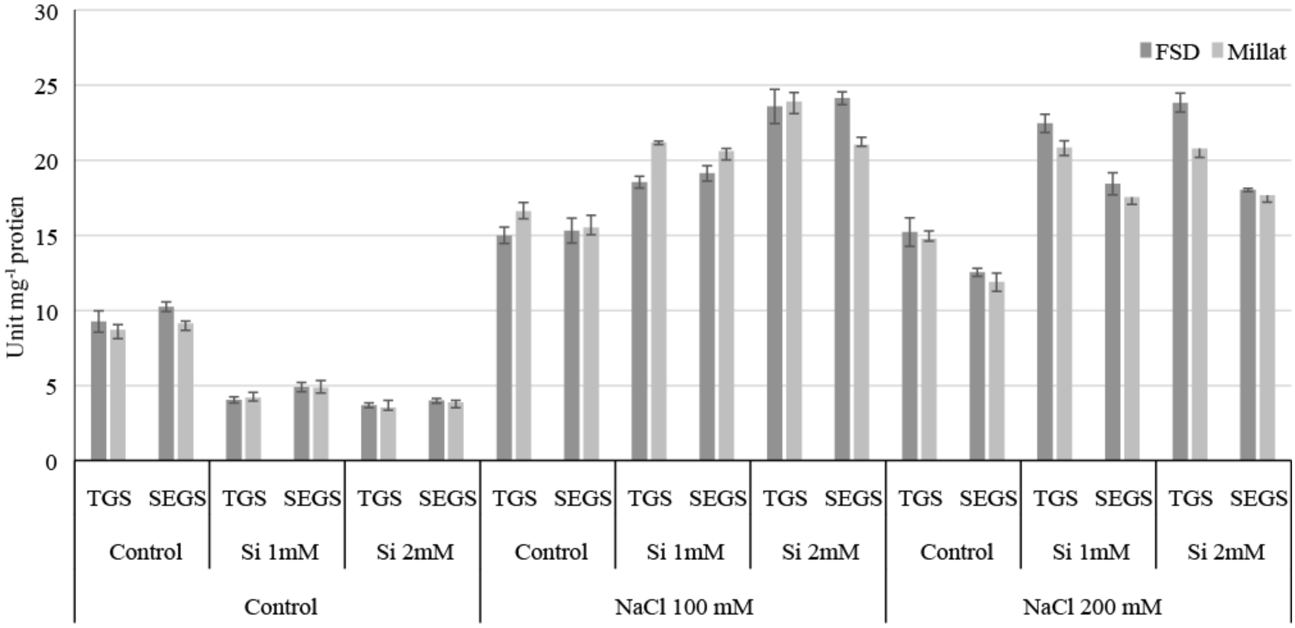

Fig. 12. Effect of Si (H_2SiO_3) on POD activity in wheat leaves at tillering (TGS) and stem elongation growth stage (SEGS) under saline condition; the values are means of three replications ± standard error.

Peroxidase (POD): The activity of peroxidase (POD) in the flag leaves was curtailed significantly when both wheat varieties were exposed to salinity stress (Fig. 12). The higher decrease was indicated by the wheat plants exposed to 200 mM NaCl. The wheat variety Millat-2011 showed more reduction in POD activity as compared to Faisalabad-2008 under both normal and saline conditions. Addition of Si profoundly increased the POD activity under stress conditions but conversely showed adverse effect in case of normal condition by reducing considerably the activity of POD. Relatively more activity of POD was observed at the tillering stage in both wheat varieties. Faisalabad-2008 showed the maximum POD activity (24.13 unit mg^{-1} protein) at the stem elongation stage in the plants treated with Si @ 2 mM in comparison with POD activity (21.02 unit mg^{-1} protein) in the shoot of Millat-2011 under saline conditions (100 mM NaCl), while the lowest POD activity (3.53 unit mg^{-1} protein) was determined in the shoot of Millat-2011 at the tillering stage in the plants treated with Si @ 2 mM under non-saline conditions. A positive correlation (R=0.46, n=36) was found between shoot Si concentration and activity of peroxidase (POD) under saline conditions (Fig. 15).

Proline: The data indicated that proline concentration was markedly increased in the leaves of both wheat varieties due to salt stress (Fig. 13). The higher values of proline contents were observed in the leaves of

Faisalabad-2008 as compared to those in Millat-2011 in all treatments. Addition of silicon (Si) profoundly decreased the proline concentration under both normal and salt stress conditions. The insignificant differences were noted between both growth stages. Faisalabad-2008 showed the maximum proline concentration (105.77) at the stem elongation stage in the plants treated with no Si in comparison with Millat-2011 (91.57) under saline conditions (200 mM NaCl) while the lowest value of proline concentration (32.52) was found in the leaves of Millat-2011 plants treated with Si at the tillering stage under non-saline conditions.

Discussion

There are number of approaches which were being used to ameliorate the adverse effects of salinity on plant growth. In the current study, silicon (Si) was used as a nutritional management to alleviate the toxic effect of sodium on wheat crop. The exogenous use of mineral nutrients has been proved to be effective to reduce salt toxicity in plants (Raza *et al.*, 2006). Yield and growth of several agricultural crops were improved by the application of Si under stress conditions (Epstein, 2001; Ma *et al.*, 2001: Ashraf *et al.* 2009, 2010). Presently, its role is well known to mitigate the adverse effects of various abiotic and biotic stresses on plant growth (Liang *et al*., 1999; Gong *et al*., 2006).

The findings of the current study regarding RGR revealed (Fig. 1) that considerable increase in relative growth rate of both varieties was observed with the addition of silicon (Si). Similarly, Yeo *et al.* (1990) reported that silicon increased growth in rice crop under salinity stress. Hattori *et al.* (2005) reported an increased growth rate of crop with the addition of silicon under drought stress. The difference in relative growth rate (RGR) between both wheat varieties in the present study may have been due to their genetic difference. The similar findings were shown by Ahmad *et al.* (2012) who reported that difference in relative growth rate between varieties might be due to adaptability measure in response to Si application and plant genetic potential.

The higher accumulation of Na^+ and Cl^- ions in wheat leaves was observed in the wheat plants treated with salinity stress (Gong *et al.*, 2006). The data (Fig. 4) also indicated that both wheat varieties i.e. Faisalabad-2008 and Millat-2011, showed significantly ($p<0.05$) higher Na^+ accumulation in their leaves under salt stress conditions. The concentration of Na^+ was significantly greater in the leaves of Millat-2011. Any phenomenon that reduces the sodium (Na^+) uptake in plant tissues enables the plant to cope with salinity stress (Raza *et al.*, 2006). Several scientists characterized wheat as a silicon accumulator (Mayland *et al.*, 1991). Hence, addition of Si to the growth medium remarkably (Fig. 2) enhanced the silicon (Si) concentration in the shoots of both wheat varieties under salt stress and normal conditions. The Si accumulation within the plant decreased considerably the sodium (Na^+) concentration in the shoot of silicon treated plants of both varieties under normal and saline conditions. The accumulation of silicon (Si) within plant bodies reduces the translocation of Na^+ from root to shoot not only by making complex with Na^+ but also by decreasing contents of Na^+ within plant tissues (Saqib *et al.*, 2008; Habibi *et al*., 2014). Silicon (Si) deposition occurs in different plant parts as in shoot epidermis but can also take place in cell wall of root endodermis (Keller *et al*., 2015). However, formation of phytoliths and their composition and localization differ among plant species (Li *et al*., 2015). Sodium (Na^+) translocation from root to shoot decreased due to transpirational inhibitory effect created by Si in the apoplast (Yeo *et al.,* 1999; Shi *et al*., 2013) after its accumulation in the root exodermis and endodermis (Gong *et al.*, 2003).

Salinity stress (Fig. 6) significantly ($p\leq0.05$**)** reduced potassium (K^+) concentration in the shoot of both wheat varieties. The higher K^+ concentration was shown by Faisalabad-2008 as compared to Millat-2011both under salt stress and normal conditions. A significant decrease in K^+ concentration in barley shoots was reported when treated with salt stress (Liang *et al.* 1999) and in alfalfa (Wang & Han, 2007). Ashraf *et al.* (2010) observed enhanced potassium (K^+) and reduced sodium (Na^+) uptake by the addition of silicon (Si) in sugarcane The increased concentration of potassium (K^+) and reduction in sodium (Na^+) uptake with Si, indicate its role to alter the flux through potassium (K^+) ion transporters, resultantly improving K^+: Na^+ ratio in the plant tissues. A significant positive correlation (R=0.50, n=36) was found between shoot Si concentration and shoot K^+: Na^+ ratio under saline conditions.

It was reported that Si application promotes selective uptake of potassium (K^+) and a large part of sodium (Na^+) was found to be present in the apoplast because of the presence of phytoliths in the apoplast (Liang *et al*., 1999; Khoshgoftarmanesh *et al*., 2014). Potassium (K^+) uptake is an energy dependent process and associated with H^+-ATPase activity present at plasma lemma. The activity of H^+-ATPase increased considerably in the presence of Si under salt stress.

Besides osmotic effect, ion toxicity and nutritional imbalance, salt stress also executes oxidative damage (Guetadahan *et al.*, 1998), through the production of ROS species such as superoxide (O_2^-), hydroxyl radical, hydrogen peroxide (H_2O_2), and singlet oxygen (Lee *et al.*, 2001). The production of ROS was reported as important mechanisms through which salinity stress causes lipid peroxidation in higher plants (Li *et al*., 2010; Fahad *et al*., 2015).These ROS species can damage proteins, nucleic acids and lipids. Plants eliminate these ROS through antioxidative scavenging systems (Menezes-Benavente *et al.*, 2004). The activity of antioxidant enzymes like catalase (CAT), superoxide dismutase (SOD), and peroxidase (POD) was inhibited by salinity stress which weakened the plant defensive system in both wheat varieties. The damage caused by salt stress was related to deposition of Na^+ in leaves followed by reduced enzyme activity and synthesis of protein (Tester & Davenport, 2003). Silicon (Si) moderately counteracts the harmful effects of salinity stress by improving the activities of CAT and SOD, as well as the synthesis of soluble proteins in tomato (Kim *et al*., 2014; Li *et al*., 2015), barley (Liang *et al*., 1996) and cucumber (Khoshgoftarmanesh *et al*., 2014). Addition of Si stimulated the scavenging system and supported the production of superoxide dismutase (SOD), peroxidase

(POD) and catalase (CAT) in both wheat varieties. The previous research indicated that Si alleviated the injurious influence of salt toxicity by improving the antioxidant enzymes activity in the plant leaves (Zhu *et al.*, 2015; Al-Aghabary *et al.*, 2004) A significant increase was also noted in the production of soluble proteins by the both wheat varieties in response to Si addition under both saline and non-saline conditions. However at normal condition the activities of antioxidants enzymes significantly decreased with the addition of silicon. Furthermore, relatively higher concentration of SOD, CAT and POD were recorded at stem elongation stage. It might be due to more accumulation of Si in plant tissues at stem elongation stage as compare to tillering stage. It was suggested that increased activities of antioxidants was associated with increased K^+ uptake and improved water status by the Si addition under stressed condition as it was noted that production ROS in the maize plants was decreased by the application of silicon (Moussa, 2006). The added silicon (Si) in wheat also enhanced the activities of antioxidants and reduced ROS creation under drought condition (Gong *et al.*, 2005). Production of SOD is considered as first line of defense against ROS which can detoxify the superoxide converting to H_2O_2 (Fridovich, 1986). Therefore, it has been concluded that addition of Si reduced the deteriorating effect of salinity by stimulating the activity of antioxidant enzymes particularly SOD activity in both wheat varieties. Comparatively Faisalabad-2008 exhibited more activities of antioxidant enzyme activity in response to Si application. A significant positive relationship was found between the shoot Si concentration and activities of SOD (R=0.66, n=36) CAT (R=0.22, n=36) and POD (R=0.46, n=36) under saline conditions (Fig. 15).

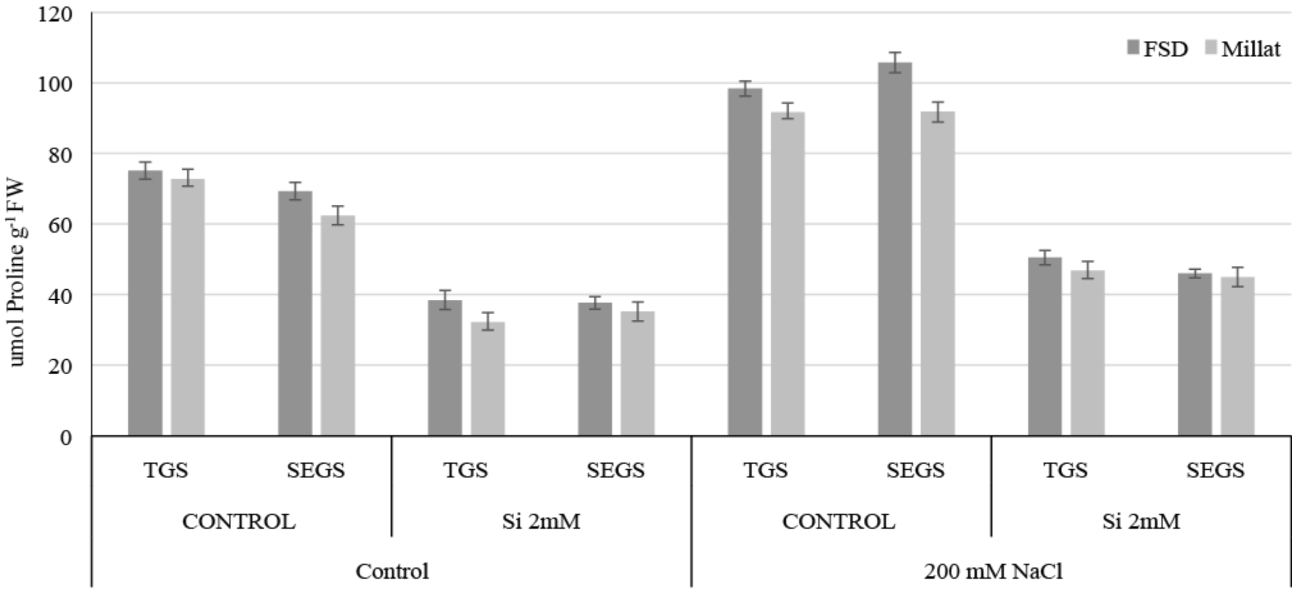

Fig. 13. Effect of Si (H_2SiO_3) on proline concentration in wheat at tillering (TGS) and stem elongation growth stage (SEGS) .under saline condition; the values are means of three replications ± standard error

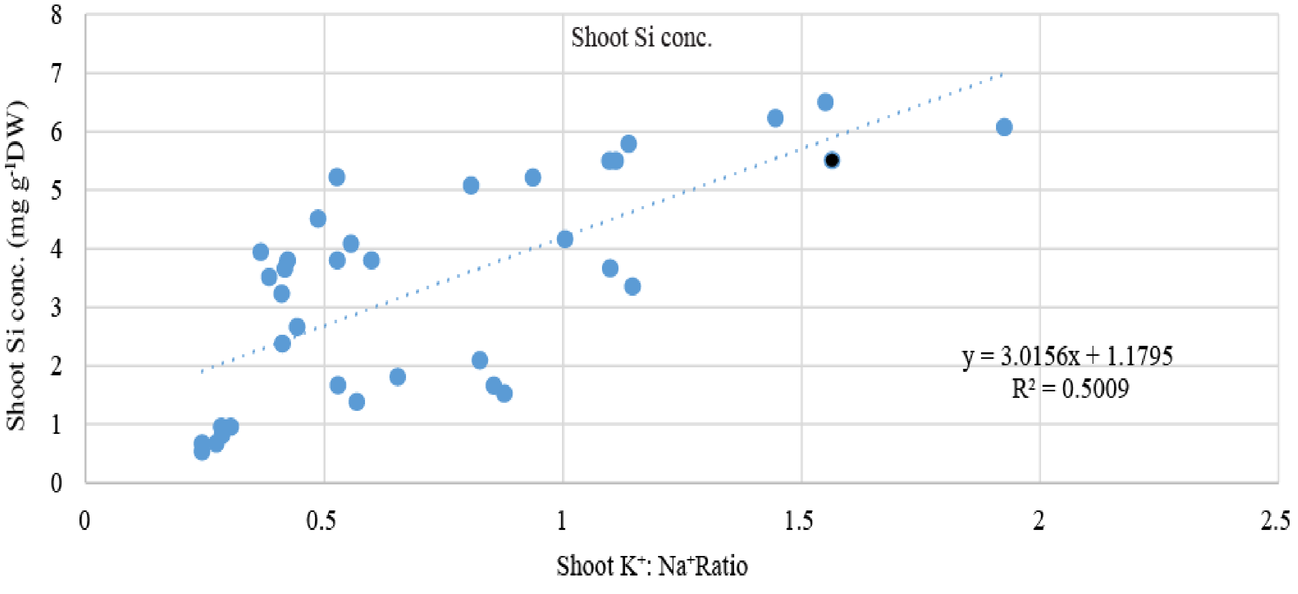

Fig. 14. Relationship between shoot Si conc. and shoot K^+: Na^+ ratio of wheat (Faisalabad-2008 & Millat-2011) treated with Si under salt stress condition (100 mM NaCl).

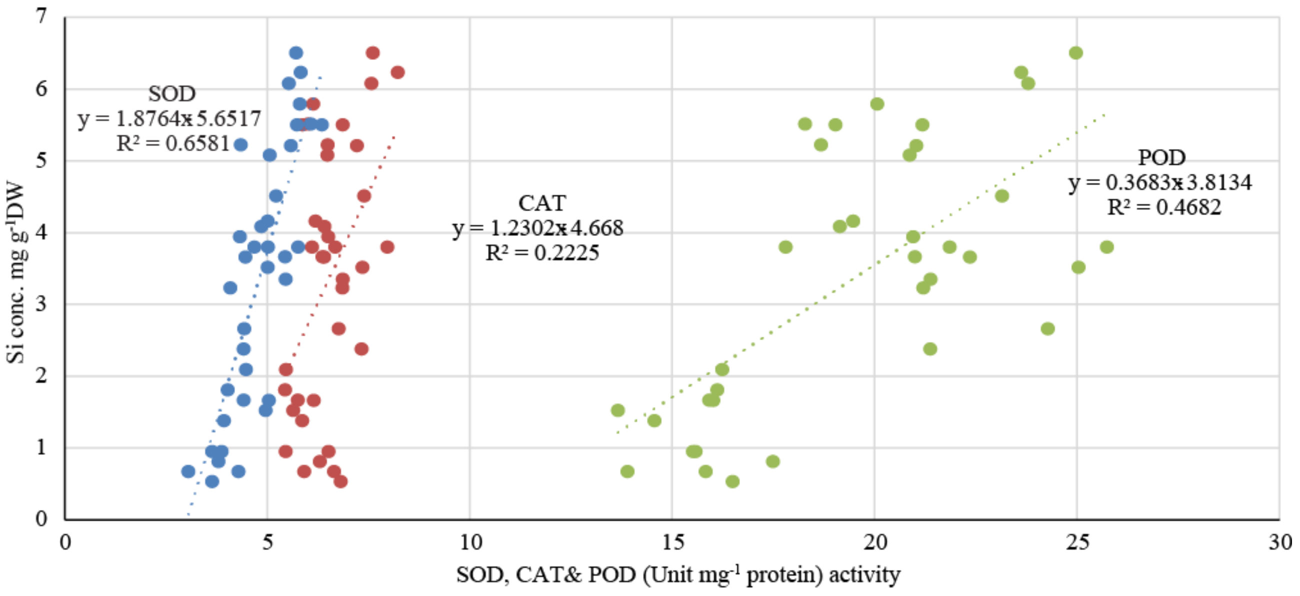

Fig. 15. Relationship between shoot Si conc. and activities of SOD, CAT & POD in the shoot of wheat plants treated with Si under salt stress condition

Under stress conditions, plants tend to produce and collect compatible solutes like proline and other low molecular weight organic solutes (Ashraf & Foolad, 2007). The data indicated that proline concentration was markedly increased in the leaves of both wheat varieties due to salt stress (Fig. 13). The higher values of proline contents were observed in the leaves of Faisalabad-2008 as compared to those in Millat-2011 in all treatments. Addition of Si profoundly decreased the proline concentration under both normal and salt stress conditions. The low proline concentration in non-saline condition and high concentration in stress conditions reported as common mechanisms of salt tolerance (Ketchum *et al.,* 1991).The proline accumulated in the root tip during stress conditions and it was suggested that it acted as a sink for excess nitrogen (Dubey & Pessarakli, 1995). The decrease in proline concentration with the addition of Si both under normal and saline conditions might have been due to formation of silaproline as reported in humans (Vivet *et al.,* 2000). The reduction in proline concentration in the leaves of salt affected plants with the application of silicon (Si) may reveal the improvement of stress damage (Zhu & Gong 2014).

Addition of Si to the growth medium enhanced K^+ uptake and decreased Na^+ uptake in both varieties, thereby enhancing K^+/Na^+ selectivity ratio in leaves. It can be assumed that wheat variety Faisalabad-2008 has a certain affinity to tolerate saline conditions while wheat variety Millet-2011 had low affinity to tolerate saline conditions. However, Si promoted growth in both varieties under saline as well as normal conditions. The Si effect was found to be time-dependent and turned into stronger with the passage of time. Thus Si has shown more positive results at the stem elongation/jointing stage as compared to the tillering stage on the basis of growth, physiological attributes, and ionic concentration, but there was no significant difference in the activity of antioxidant enzyme at both stages.

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(Received for publication 11 April 2016)