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Original Article

Alleviation of Salinity Stress in Maize Using Silicon Nutrition

Muhammad Mohsin RAZA[1], Sami ULLAH[2], Tariq AZIZ[3], Tanveer ABBAS[2], Malik Muhammad YOUSAF[1], Volkan ALTAY[4], Munir OZTURK[5]*

[1]Pakistan Agriculture Research Council, Arid Zone Research Institute Bahawalpur, Pakistan; mohsinraza_5@yahoo.com; malikparc@gmail.com
[2]Institute of Agricultural Resources and Regional Planning Chinese Academy of Agricultural Sciences Beijing 100081, China; Samisheen14@yahoo.com; abbastanveer272@gmail.com
[3]Institute of Soil and Environmental Sciences University of Agriculture Faisalabad, Pakistan; draziz@edu.uaf.pk
[4]Hatay Mustafa Kemal University, Faculty of Science and Arts, Biology Department, Hatay, Turkey; volkanaltay34@gmail.com
*[5]Ege University, Science Faculty, Botany Department, Bornova, Izmir, Turkey; munirozturk@gmail.com (*corresponding author)*

Abstract

Improving salinity tolerance through mineral nutrition in plants is emerging strategy for sustainable agriculture under limited resources. Silicon (Si) is considered as silver bullet to mitigate biotic and abiotic stresses. Present study was conducted to understand the new mechanisms of Si nutrition against salinity stress in two different maize (*Zea mays* L.) cultivars ('Syngenta-8441' and 'Pearl'). Three different levels of NaCl (0.67, 8 and 13 dSm^{-1}) were used with and without addition of silicic acid (2 mM). Distilled water was used for irrigation purposes and crop was harvested after 40 days of post germination. Results indicated that plant biomass reduced under saline condition while Si application increased growth parameters. Data regarding chemical analysis showed that Si nutrition reduced Na^+ concentration and enhanced K^+ levels in root, shoot, new and old leaves of 'Sygenta 8441' compared to 'Pearl' maize variety. Si application improved both chlorophyll a and b in both maize cultivars compared to NaCl-treated plants. The current findings indicate that Si nutrition can alleviate salinity stress in maize without decreasing growth attributes of crop and 'Sygenta 8441' is a salt resistant variety whereas 'Pearl' is a salt sensitive variety.

Keywords: alleviation; plant growth; salinity; silicon nutrition; *Zea mays*

Introduction

Food security is a serious issue in developing countries with ever-increasing population. Recent estimates show that in 2017, 821 million people have suffered from under nourishment due to poverty and/or natural calamities (FAO, 2018). Land degradation occurs mainly due to various abiotic factors like drought, salinity, and pollution. Among various abiotic stresses, soil salinity poses a serious threat to the agriculture around the globe (Ghassemi *et al*., 1995; Ozturk *et al*., 2006; Chedlly *et al*., 2008; Ashraf *et al*., 2012; Hakeem *et al*., 2013; Hameed *et al*., 2019). Salinity is associated with ion toxicity and physiological drought to plants (Acosta-Motos *et al*., 2015). In most cases, Na^+ and Cl^- ions are the cause of salinity. The Na^+ and Cl^- induce salinity stress which usually leads to reduced plant growth rate together with nutrition imbalance, and at higher concentrations cause leaf scorching, chlorosis and necrosis (Munns and Tester, 2008; Ashraf *et al*., 2010). Salt stress condition affects physiology of plants by changing water and ionic balance of plant cells, nitrogen and carbon dioxide assimilation rates as well as protein biosynthesis mechanisms (Cusido *et al*., 1987; Shannon and Grieve, 1999; Wahid *et al*., 1999; Hasegawa *et al*., 2000). Yield, plant biomass as well as quality of the product are severely affected negatively due to instability of plant between organic and inorganic ingredients (Gunes *et al*., 1996, 2007; Nasim *et al*., 2008). Reduced plant growth due to salt-stressed condition depends upon amount of salt present in soil, type of specific salt and plant affected plant tissues (Munns and Tester, 2008). Mostly excess salt concentration species (Na^+ and Cl) damage plant root membranes effecting selectivity mechanism of the membrane followed by an adverse effect on potassium balance, decrease in nitrogen assimilation enzyme activity (nitrate reductase), inhibition of photo system II (Gadallah, 1999) and chlorophyll breakdown

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(Ball *et al*., 1987, Hasegawa *et al*., 2000). Judicious use of mineral nutrition such as high potassic fertilizers (Raza *et al*., 2006) and osmo-protectant applications (Arfan *et al*., 2007) are healthy strategies as these strengthen the plant to cope against salt stress but cannot be used for long time for permanent amelioration. Some researchers have also used hormones like abscisic acid (ABA) and natural acid including jasmonic acid (JA) for higher growth and yield under salt stressed condition, but these approaches are not feasible for common farmers due to the expensiveness of hormones and acids (Gautam and Singh, 2009; Hayat *et al*., 2010; Singh and Gautam, 2013).

Silicon (Si) has considerable ameliorative features against Na^+ and Cl^- toxicity, as it allows plants to grow more effectively on saline soils, which appears to be of economic gain primarily for industrializing countries. It improves plant growth especially under abiotic stresses and reduces radiation effects as well as water loss up to 30 percent (Dionisio-Sese and Tobita, 1998). Si uptake by plant under salinity increases root activity and inhibits transpiration but increases the activity of ATPase and PPase in plasma membrane, there by leading to an increase in the K and decreases Na uptake (Liang *et al*., 2007; Khan *et al*., 2018).

In Pakistan, maize (*Zea mays* L.) is an important profitable crop after potato and its growth and yield are highly retarded as a glycophyte as the soil salinity increases. However, availability of Si in the growth medium hinders the salinity effect by changing soil/plant factors, but specific mechanisms are still debatable (Tariq and Iqbal, 2010; Kafi and Rahimi, 2011; Khan *et al*., 2003, 2018). Data on the role of Si in alleviating the salinity stress effects on maize has not been explained at length. Silicon nutrition management increases growth of maize following salt stress. Present study was planned with following objectives: 1) to compare the two maize cultivars ('Syngenta-8441' and 'Pearl') against salinity stress and evaluate the role of Si in improving salinity stress tolerance, 2) to investigate the Si effects on ionic concentration under salinity stressed maize plants.

Materials and Methods

Experimental details

A portion of prepared soil was analysed for various physico-chemical characteristics (Table 1) according to the U.S. salinity Lab. Staff (1954). Ten kg polyethylene lined plastic pots were filled with soil and whole experiment was set under completely randomized design with factorial arrangement. Chemical fertilizers were applied as N, P and K 270-160-90 kg ha^{-1}, before sowing as urea, diammonium phosphate and single super phosphate respectively. Natural salt NaCl was applied at 0 and 60 mM in solution form for maintaining salinity levels. After fertigation of NPK fertilizers and salt, the soil in each pot was remixed to make it homogenized. Silicic acid was applied at different rates (0 and 2 mM) as Si source and mixed well in each pot. 'Syngenta-8441'-salt tolerant and 'Pearl'-salt sensitive maize cultivars were selected for this experiment and distilled water was applied as irrigation water whenever needed. Ten seeds were sown in each pot and after successful germination; six plants out of ten were uprooted and incorporated into the soil of same pot.

Agronomic and physiological parameters

Agronomic parameters of maize including root and shoot fresh weight; root and shoot dry weight, plant height and plant weight were calculated by following standard procedures (Naveed *et al*., 2014). Fresh weight was measured after harvesting while dry weight was computed after oven drying until constant weight with electronic balance. Chlorophyll a and b were measured by the method outlined by Strain and Svec (1966). Upper 3rd fully expanded leaf was selected and harvested after 40 days of germination, 1 g of this was ground in 90 percent acetone manually using pestle and mortar, absorbance was measured with UV/visible spectrophotometer (Shimadzu, UV-1201, Kyoto, Japan) and chlorophyll concentration calculated as given by Khan *et al*. (2018).

Plant chemical analysis

The plants harvested after 40 days of germination were washed twice with distilled water, blotted dry with tissue paper and subjected to chemical analysis. Samples were air-dried and then oven dried at 65 °C for two days till constant weight. For determination of Na^+ and K^+ concentrations, samples were grinded with the help of grinder (IKA Werke). Both were measured in the root, shoot, old and young leaves using flame photometer (Jenway PFP-7, England) and concentrations calculated by calibration curve (Naveed *et al*., 2014). For Si determination 0.5 g grounded samples were digested in 2 mL 50 percent hydrogen peroxide (H_2O_2) and 4.5 g 50 percent NaOH in teflon beakers on a hot plate at 150 °C for 4 hours. Silicon concentration was measured as given by Elliot and Synder (1991) and absorbance measured at 650 nm wave length with a UV visible spectrophotometer (Shimdzu, Spectronic 100, Japan) (Ali *et al*., 2012).

Statistical analysis

Main and interactive effects of silicon, salinity and genetic variability were analyzed on various response variables by analysis of variance (ANOVA) technique. Least significant difference (LSD) test was used to determine significantly different treatment means (Steel *et al*., 1997). Statistix 8.1® (Analytical Software, Tallahassee, USA) and Microsoft Excel® (Microsoft Cooperation, USA) were used for statistical analysis.

Results and Discussion

Agronomic and physiological parameters

Data regarding agronomic parameters has shown that Si application improves plant growth while salinity suppresses the plant growth parameters. Data has revealed that salinity stress decreased plant fresh weight in 'Pearl' variety up to 28 g as compared to the controls (87.33 g), but Si application alone increased plant weight up to 107 g. Similar trend was observed for 'Sygenta 8441' with minute differences. The dry plant weight has shown the same trend as fresh weight in both maize varieties. Root fresh and dry weight was non-significant in main as well as in interaction effects, but Si application improved it as compared to salt stress condition (Fig. 1). Maximum and minimum plant height (65 and 28.78 cm) was recorded with Si applied singly and under

Table 1. Physico-chemical properties of soil used for experiment before amendment

pHs	7.89
Electrical Conductivity (ECs) (dS m^{-1})	0.67
Cation Exchange Capacity (CEC) ($cmol_c$ kg^{-1})	14.80
Cl^- ($mmol_c$ L^{-1})	13.60
SO_4^{-2} ($mmol_c$ L^{-1})	10.11
Ca^{+2} + Mg^{+2} ($mmol_c$ L^{-1})	8.60
Na^+ ($mmol_c$ L^{-1})	15.68
Sodium Absorption Rate (SAR) (mmol L^{-1})$^{1/2}$	7.76
Organic Matter (OM) (%)	0.85
N (%)	0.03
Olsen's P (mg kg^{-1})	7.80
K (mg kg^{-1})	158

salt stressed condition in 'Sygenta' variety. A combined application of Si and natural salt has improved plant height up to 44.87 and 42 cm in 'Sygenta' and 'Pearl' varieties respectively and it was significantly higher than salt stressed condition (Fig. 2). The root length data shows that Si application significantly improves root length in both maize varieties as compared to either in saline condition or combined application of Si and salt. Salinity stress reduces both chlorophyll a and b in both maize verities compared to Si applied singly or as Si+NaCl. In the case of chlorophyll a maximum value was recorded from Si applied singly (0.49 ug g^{-1}) followed by control for 'Pearl', same trend was observed for 'Sygenta' variety. Si+NaCl application has improved chlorophyll b up to 0.70 ug g^{-1} in 'Sygenta', which was non-significant to Si applied singly and control treatments; but in 'Pearl' variety Si application applied singly has enhanced chlorophyll b up to 0.80 µg g^{-1}. Salinity has reduced all growth and physiological maize attributes of both cultivars but Si application has improved all these parameters even under salt stressed condition (Fig. 3).

Salinity stresses negatively affect plant growth and yield. NaCl is the major salt in salt affected soils, which not only reduces population of plants in the field but also decreases plant biomass and increases production cost in such areas. Maize crop is more sensitive to salinity at early growth stages compared to later stages. In this experiment all the growth parameters including plant biomass, plant height and root length decreased under saline condition but enhanced when the experimental plants were Si- treated. Our findings are in full agreement with those published by Moussa (2005), Kafi and Rahimi (2011) and Parveen and Ashraf (2010). The possible mechanism may be the reduction in cell membrane integrity under salt stressed condition (Marschner, 1995). Interestingly, no significant effect was observed in the case of dry matter yield in both varieties (Fig. 1), but possible mechanism may vary from genotype to genotype even within a species. Plant biomass and plant height might be reduced due to less cell expansion under salt stressed condition due to the presence of Na^+ in soil solution (Khan *et al.*, 2018). Si taken by plants acts as detoxicant against Na^+ in cell wall of plant and binds to the cell wall instead of Na^+ resulting in a decrease in its movement towards shoots and leaves (Khan *et al.*, 2017, 2018). Both chlorophyll a and b measured here were less under salt stressed condition compared to Si application treatment. The decrease in chlorophyll concentration might be due to improper working of photosynthetic mechanism (Mateos-Naranjo *et al.*, 2013) or may be due to production of destructive enzymes such as chlorophyllase produced in the plants under stressed conditions, which leads to the degradation of chlorophyll apparatus (Sabater and Rodriquez, 1978) or due to production of reactive oxygen species (Moussa, 2005). Si application has improved chlorophyll levels as recorded in this study, because it might have detoxifying effect on ROS (Fig. 2). Our results are in full agreement with those of Wang *et al.* (2015).

Ionic concentration

The results regarding the chemical analysis are more prominent than growth parameters following application with or without Si. The concentrations of potassium and sodium were measured in young and old leaves as well as in root and shoot. In young leaves maximum K concentration (0.56 mg g^{-1}) was recorded in 'Sygenta 8441' variety with just Si application and it was greater than even control treatment. Si+NaCl application did not improve K concentration significantly in young leaves compared to salt stress condition in both cultivars. In older leaves, K concentration difference in main and interaction effects was non-significant for all treatments in both cultivars (Fig. 4). The results were reverse regarding K concentration in root of both verities. Salinity reduced K uptake in root while Si application enhanced K levels in Si treatment given alone and Si+NaCl applied up to 12.10 and 10.16 mg g^{-1} in 'Pearl' variety; similar trend was recorded for other maize variety with small differences. Na uptake was also significantly reduced with Si application in both verities, but most prominent effect was observed in the 'Pearl' compared to 'Sygenta 8441'. Minimum Na level in young and old leaves (0.08 and 0.09 mg g^{-1}) was recorded in control treatment (no salt application) followed by Si applied alone in 'Sygenta' and 'Pearl' varieties respectively, both treatments were statistically non-significant. Maximum Na concentration was found in the treatment where NaCl was applied alone in both old and young leaves in both verities. Comparatively more Na concentration was recorded in root as compared to shoot under salt stress condition in both the varieties compared to young and old leaves. With Si application Na concentration in roots was reduced up to 50 percent compared to saline condition in 'Sygenta 8841' variety; Na concentration decreased by 43 percent compared to the control treatment.

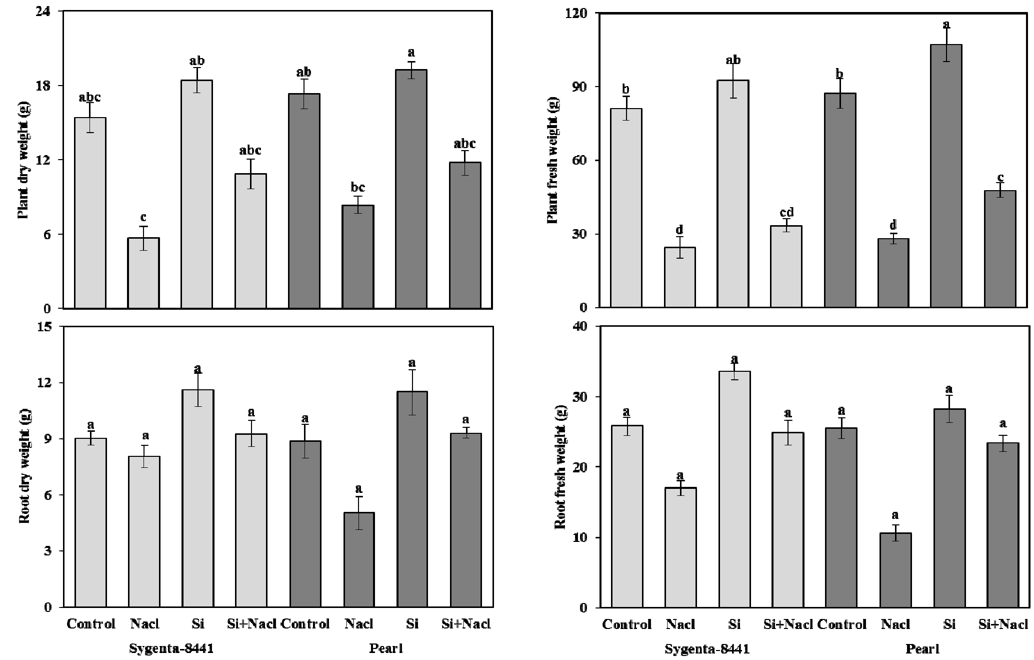

Fig. 1. Effect of silicon and salinity on maize shoot and root fresh and dry weight

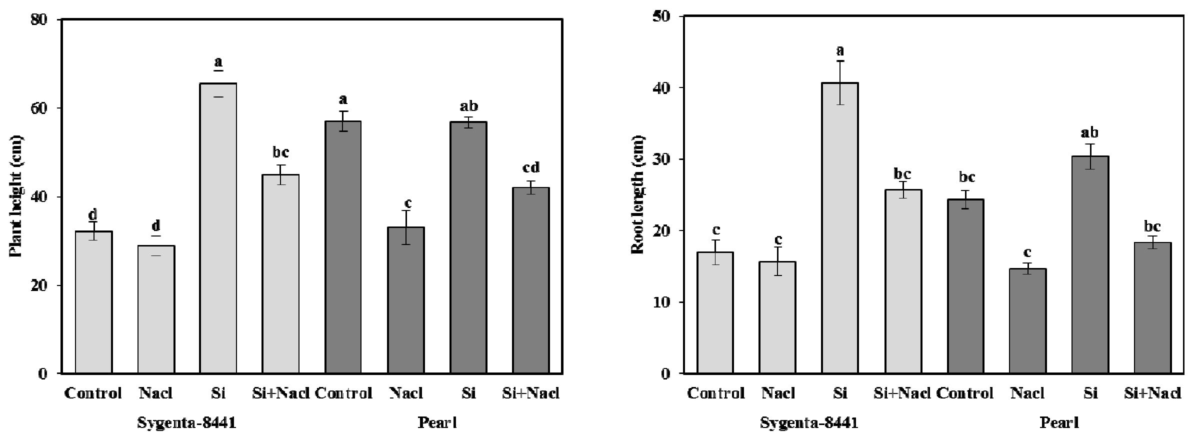

Fig. 2. Plant height influenced by silicon nutrition under salt stressed condition

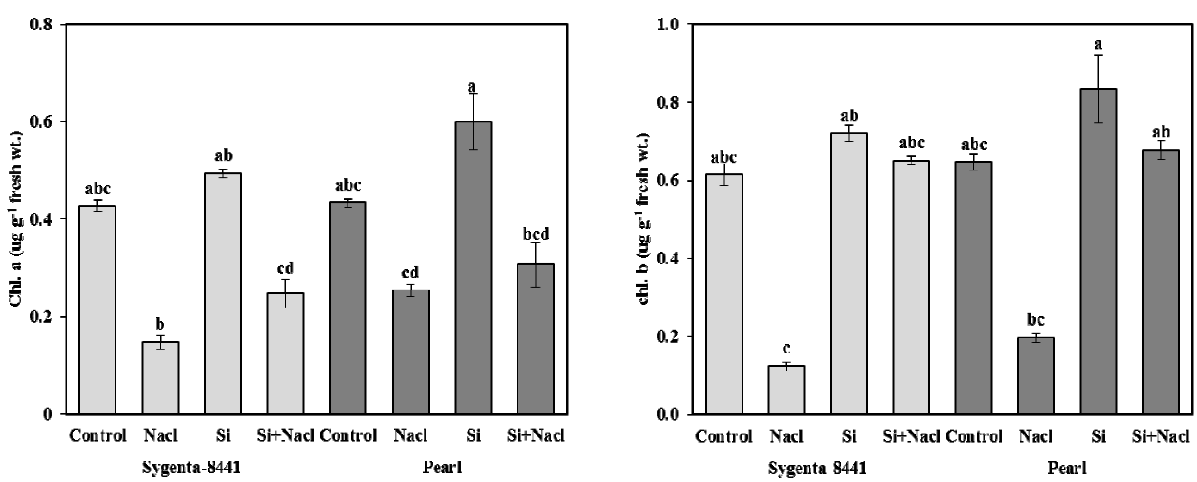

Fig. 3. Effect of silicon nutrition and salinity on chlorophyll content

Na concentration in the roots of 'Pearl' variety was higher compared to 'Sygenta 8441' under salt stressed condition. Similar trend was recorded for Na concentration in shoot in both maize cultivars with minor differences (Fig. 5).

Si concentration in shoot as well as root was statistically different due to application of Si in both verities. Maximum Si (8.33 mg kg^{-1}) was monitored in 'Sygenta 8441' variety where Si was applied singly followed by Si+NaCl application. Under salt stressed condition 1.3 and 1.7 mg kg^{-1} Si were recorded in shoot of 'Sygenta 8441' and 'Pearl' varieties respectively. Si concentration in roots was higher compared to shoots in both varieties for salt stressed as well as other treatments. Si+NaCl combined application increased Si level in root up to 8 and 7.66 mg kg^{-1} root dry weight and these values were statistically non-significant for both maize cultivars when Si was applied singly (Fig. 6).

Na concentration was higher under salt stressed soil condition and K is low in the plants grown on saline soils. The basic mechanism behind this might be less integrity of plant cell membrane resulting in leaking of K ions while maintaining Na ions in plant body (Marschner, 1995). The increase of Na concentration either in leaves, roots or shoots damages cells and ultimately causes cell death (Parida and Das, 2005). In our experiment salt tolerance maize variety 'Sygenta 8441' and salt sensitive variety 'Pearl' have shown that ionic Na concentration was higher in 'Pearl' as compared less Na concentration recorded in 'Sygenta 8441'. It is well known now that salt resistant verities accumulate less Na in tissues and our findings coincide with these results (Aktas *et al*., 2006; Khan *et al*., 2017). Si-treated plants have shown more promising results regarding growth and other parameters compared to salt stressed plants. Si present in the plant protects them against physical injury as well as cell destructing ions (Na^+) which are converted into more stable form under salt stress condition (Khan *et al*., 2018). Low concentration of reactive oxygen species (ROS) are necessary for plant metabolism and signaling but higher concentration causes autocatalysis of lipid membranes (Xu *et al*., 2006; Choudhury *et al*., 2013). Under salt stressed condition, more ROS is produced inside the plant resulting in weakening of cell membrane. Si application induces salinity tolerance in plants probably via regulation of ROS under salt stressed condition (Zhang *et al*., 2006; Rouhier and Jacquot, 2008). In this study, higher Na concentration was noted in salt stressed plants compared to Si or Si-NaCl treated plants (Fig. 5). Therefore, we conclude that under salt stressed condition excessive amount of NaCl in soil-plant system leads to a reduction in the intra cellular water, as such compatible solutes including organic as well as inorganic solutes accumulate in cytoplasm of cell to maintain turgidity of cell resulting in higher concentration of Na^+ and Cl^- in shoot (Ahmad and Prasad, 2011).

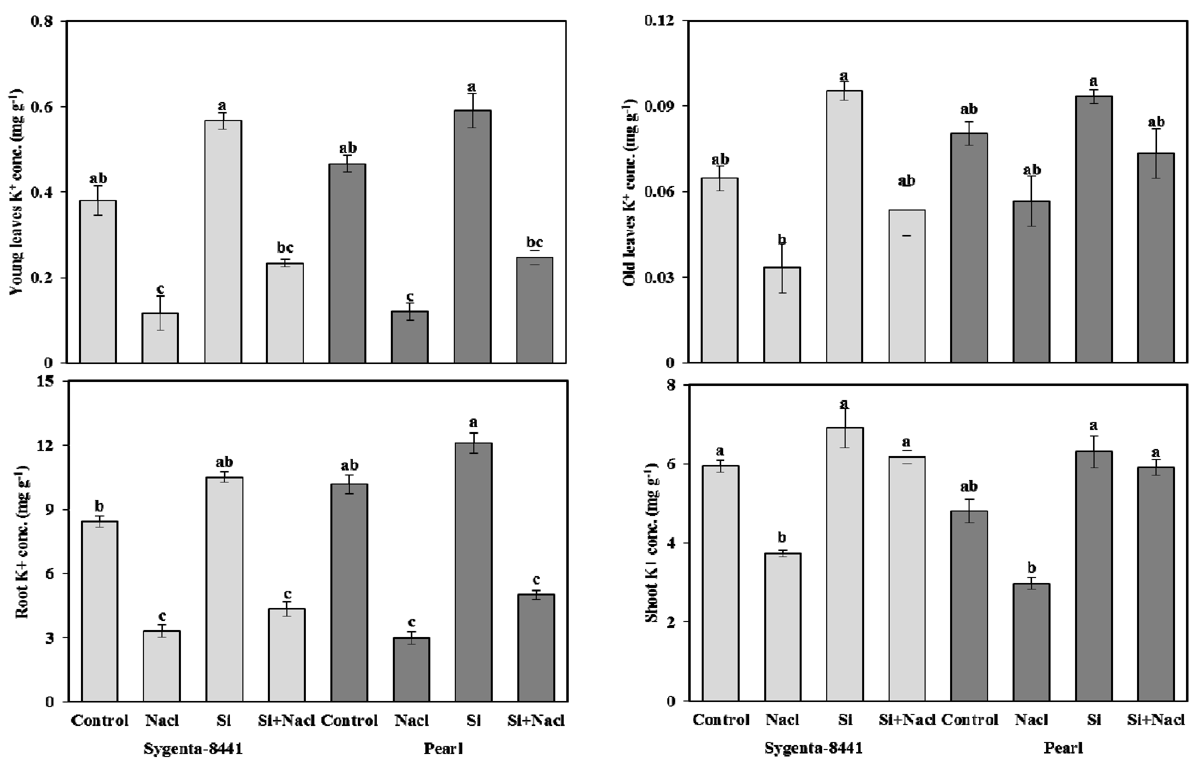

Fig. 4. Concentration of K^+ in root, shoot and leaves as affected by silicon application under salinity condition

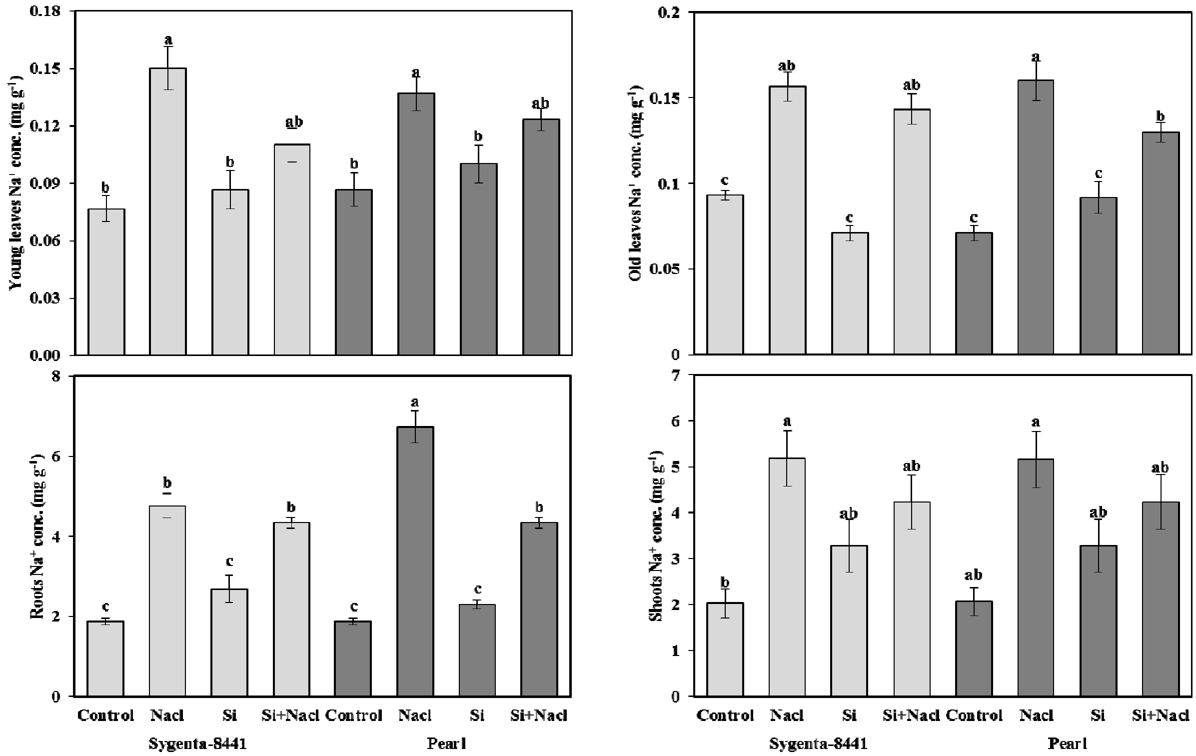

Fig. 5. Concentration of Na^{+} in root, shoot and leaves as affected by silicon application under salinity condition

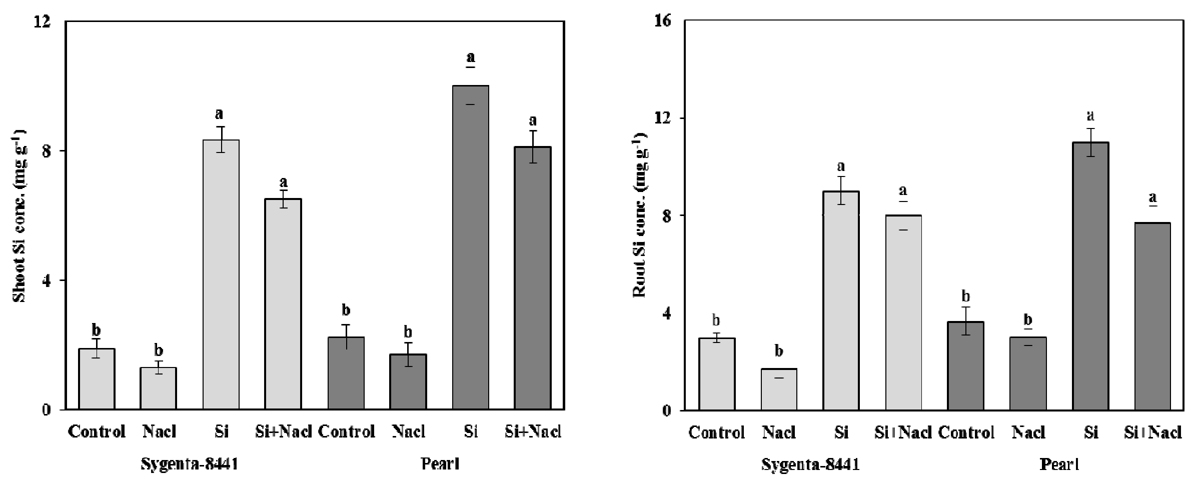

Fig. 6. Silicon concentration in root and shoot of maize plant under salt stressed environment

Conclusions

The area of salt affected soils is increasing day by day due to various reasons. These are resulting in the decrease in crop growth and yield. In the present study, NaCl has been observed to affect both maize varieties ('Sygenta 8441' and 'Pearl') negatively, with little or more difference. However, Si application has enhanced growth of plants. Si applied singly has proved more prominent compared to Si-NaCl treated plants, but both treatments were statistically significant than NaCl applied alone.

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Conflict of Interest

The authors declare that there are no conflicts of interest related to this article.

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