

SILICON APPLICATION IMPROVES Fe AND Zn USE EFFICIENCY AND GROWTH OF MAIZE GENOTYPES UNDER SALINE CONDITIONS

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Salinity causes decrease in maize growth and production. Maize occupies an important position in fodder and food crops of Pakistan. Recently, maize has been designated as silicon (Si) accumulator which can alleviate the salinity damage, a major constraint to agricultural crop production. With the objective to combat salinity stress in maize by Si applications using silicic acid $\text{Si}(\text{OH})_4$, an experiment was conducted on two contrasting maize genotypes (salt sensitive; Pak-Afgoe and salt tolerant; Ev-5098), under normal and saline conditions. Three different levels of Si (0, 1 and 3 mM) were optimized for salinity tolerance on the basis of plant morphological characters especially dry weight in hydroponics. These Si-level was further used to investigate its effect on maize in hydroponic (0 mM NaCl) and saline (100 mM NaCl). The evaluation was done on the basis of various morphological, physiological, biochemical and growth traits during the experiments. Silicon supplementation into the solution culture significantly improved the $\text{K}^+:\text{Na}^+$ with reduced Na^+ and increased K^+ uptake. Plant water relations with higher water potential, increase in chlorophyll fractions and its ratios, enhanced stomatal conductance. It was concluded that Ev-5098 is better than Pak-Afgoe under salt stress and silicon inclusion into the any growth medium is beneficial for maize and can improve crop growth by maintaining plant water status, better $\text{K}^+:\text{Na}^+$ and recovering the plant defense system adversely influenced by salt stress.

Key words: Salinity, maize, silicic acid, morphological, physiological, biochemical

INTRODUCTION

Salt-affected areas are inhabited by people in worldwide but with few opportunities of food and livelihood (Gregorio *et al.*, 2002). Almost 800 million hectare (mha) of land throughout the world is salt-affected either by salinity (397 mha) and/or by sodicity (434 mha) (FAO, 2005). Excessive accumulation of salts in soils of arid and semi arid regions are a potential factor for limiting productivity and it is a consistent process in these areas of the world (Noreen and Ashraf, 2008).

Soil salinity is a major stress that affects plant growth (Allakhverdiev *et al.*, 2000). It affects crops both by osmotic effect and by specific ion effect. Salinity reduces the water uptake and uptake of different nutrients by the plants and causes ion toxicity (Saqib *et al.*, 2008). High salts disturb the ionic homeostasis resulting in ionic toxicity, osmotic stress and increased production of reactive oxygen species (Munns, 2002). Reactive oxygen species affect many cellular activities causing oxidative damage to lipids, proteins and nucleotides. The activity of antioxidative enzymes such as catalase (CAT), peroxidase (POD), glutathione reductase (GR), and superoxide dismutase are increased under salt stress and a relationship between increased activities of enzymes and salt tolerance of plants has been reported (Mittova *et al.*, 2004).

Fouly, (1983) found that the availability of micronutrients such as Fe and Zn is much affected by pH, CaCO_3 content and soil texture. Each element of these micronutrients has its own function in plant growth. Potarzycki and Grzebisz (2009) reported that zinc exerts a great influence on basic plant life processes, such as (i) nitrogen metabolism— uptake of nitrogen and protein quality; (ii) photosynthesis— chlorophyll synthesis, carbon anhydrase activity. The previous researches clearly indicated that Zn-deficient plants showed reduced rate of protein synthesis and protein content. Gurmani, (1988) studied the response of yield and yield components of rice to Fe, Mn, Zn and Cu and concluded that Zn alone, Mn alone and combined application of Mn and Cu increased the yield significantly over NPK. Also, Zn, Mn and Cu increased yield by 15, 11 and 10% over NPK, respectively. Though the solubility of silicate minerals vary under different soil and environmental conditions, however, its concentrations in soil solutions usually range from 0.1 to 0.6 mM (Joseph, 2009).

Among all the micronutrients assimilated by plants, silicon alone is consistently present at concentrations similar to those of the macronutrients. Its concentrations in different plants range from 0.1% (similar to P and S) to more than 10% of whole plant dry matter (Epstein, 1999). Hydrated amorphous silicon compounds are likely to be deposited in different cellular parts such as cell lumens, cell walls and

intercellular spaces, its deposition below and above of the cuticle layer has also been reported. Silicon is an important micronutrient for healthy and competitive growth of all cereals including rice in Asia (Brunings, 2009). Role of silicon in plant health and growth has been investigated in silicon accumulating crops and it seemed significantly effecting (Jinab, 2008). Research evidences proved that adequate uptake of silicon can increase the tolerance of agronomic crops especially rice to both abiotic and biotic stresses (Ma and Takahashi, 2002).

Effects of silicon on yield are related to the deposition of the element under the leaf epidermis which results a physical mechanism of defense, reduces lodging, increases photosynthesis capacity and decreases transpiration losses (Korndorfer, 2004). The deficiency of micronutrients is mostly dominant under saline conditions because of increase in pH and highly concentration of dominant cations like Na^+ , Mg and Ca which have a inhibiting effect on the availability of Zn and Fe. This study conducted with the objective to study the effect of Si application on Fe and Zn acquisition and utilization by maize under salt-affected conditions. Although various studies have evaluated various aspects of these micronutrients, but it seems more accurate comment would require repeated experiments. Therefore, the present study was conducted to explore the effect of Fe, Zn and Si on yield and yield components of maize genotypes.

MATERIAL AND METHODS

Two maize (*Zea mays* L.) genotypes were used in this hydroponic experiment. Seeds of each cultivar were sterilized in HgCl_2 solution (0.1%) for 10 min and after rinsing these seeds with distilled water they were sown in plastic pots containing well washed gravels. The seedlings were allowed to grow for 15 days. Uniform sized seedlings were transferred to a hydroponic system comprising of plastic tubs filled with Hoagland's nutrient solution (half strength). The composition of the basic nutrient media was (g L^{-1}): $(\text{NH}_4)_2\text{SO}_4$ 48.2, MgSO_4 65.9, K_2SO_4 15.9, KNO_3 18.5, Ca $(\text{NO}_3)_2$ 59.9, KH_2PO_4 24.8, Fe citrate 5, $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ 0.9, $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ 0.11, $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ 0.04, H_2BO_3 2.9, H_2MoO_4 0.01 and the pH was 6.0 ± 0.5 . This solution was covered with thermo-pore white sheet having holes. The plants were supported in the holes of thermo-pore sheets. Each tub was supplied with 100 liter Hoagland's nutrient solution. The treatments in this experiment include different levels of NaCl and silicon. The concentrations of silicon used will be 0, 1 and 3.0 mM in Hoagland nutrient solution with 0 or 100 mM NaCl (salinity treatments was the same as in the first experiment). These treatments were developed by adding sodium chloride (NaCl) and silicic acid $\text{Si}(\text{OH})_4$ to the nutrient solution after four days of transplantation to the hydroponic system. The pH of the treatment solution was maintained between 5.5 and 6.5 by

using dilute NaOH or HCl solutions. The treatment solutions in all the tubs were aerated by bubbling air in the nutrient solutions. There were four replication of each treatment. The plants were allowed to grow in the treatment solutions for 28 days and at harvest the data regarding root/shoot fresh and dry weights were recorded. The oven dried shoot samples was analyzed for ion contents including Zn, Fe, Na^+ and K^+ . Si concentration was measured using calorimetric amino molybdate blue color method (Elliot and Synder, 1991) and the absorbance was measured on spectrophotometer. Zn and Fe measured on atomic absorption spectrophotometer while Na^+ and K^+ were measured on flame photometer. Chlorophyll contents were measured before harvesting by SPAD chlorophyll meter (Minolta, Japan). A fully expanded flag leaf was excised at booting stage to determine the leaf water potential. Leaf water potential was measured with water potential apparatus (Chas W. Cook & Sons, Birmingham B 42, ITT England)) following the method described by Scholander *et al.* (1964). The stomatal conductance, Photosynthetic Rate and Transpiration Rate were measured using an open system LCA-4 ADC portable infrared gas analyzer (Analytical Development Company, Hoddeson, England). The data collected was analyzed using CRD design with 3 factor arrangement.

RESULTS

Shoot and root growth: The increasing levels of salinity resulted in the corresponding decline in shoot fresh and dry weights of both the maize genotype but application of Si ameliorate the effect of salinity (Fig. 1 & 2). The difference among genotypes, Si application, salinity and their interaction was significant ($P < 0.05$) for both these parameters. In control treatment, genotypes did not differ significantly with the application of Si however in the saline treatments they differed significantly from each other and this difference widened with more salt concentration in the growing medium and also due to beneficial effect of Si application as nutrient. Due to application of Si @ 3mM under 100mM NaCl level of salinity 49.8 % increase in shoot fresh weight of tolerant genotype was observed as compared with out Si application at same level of salinity. Like shoot growth salinity resulted in the corresponding decline in the root growth as well but Si application minimized the effect of salinity. The main effects as well as their interaction were significant for both root fresh and dry weights. Reduction in fresh and dry weights of root was more in the case of salt sensitive than salt tolerant genotypes because of more Si uptake of salt tolerant genotypes under salinity.

Leaf ionic composition: Salinity significantly ($P < 0.05$) increased the leaf Na^+ concentration in both genotypes (Fig. 5) while application of Si hinder the uptake of Na^+ due to ionic competition in solution. The individual as well as

interactive effects of salinity, Silicon and genotypes were found significant.

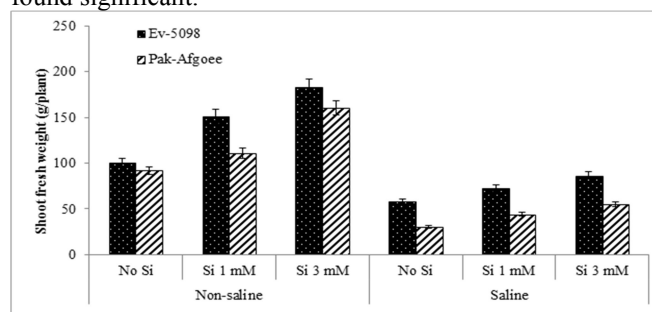


Figure 1. Effect of Si application on shoot fresh weight of Maize genotypes under non- saline and saline conditions

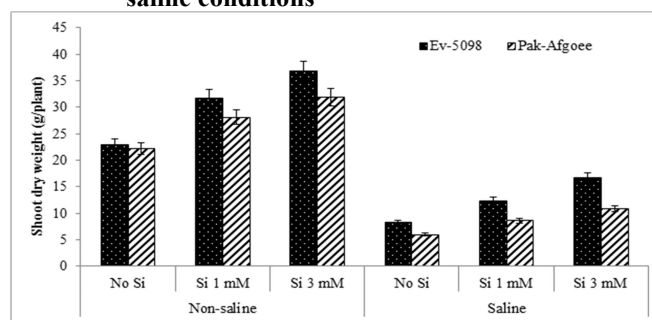


Figure 2. Effect of Si application on shoot dry weight of Maize genotypes under non- saline and saline conditions

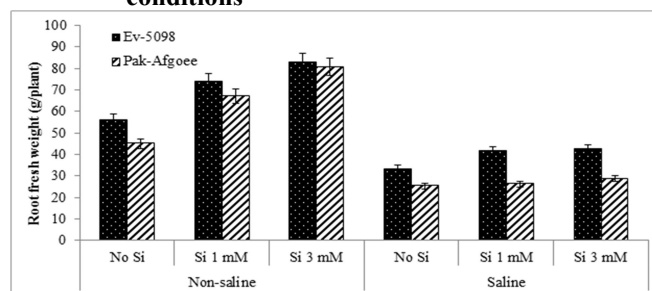


Figure 3. Effect of Si application on root fresh weight of Maize genotypes under non- saline and saline conditions

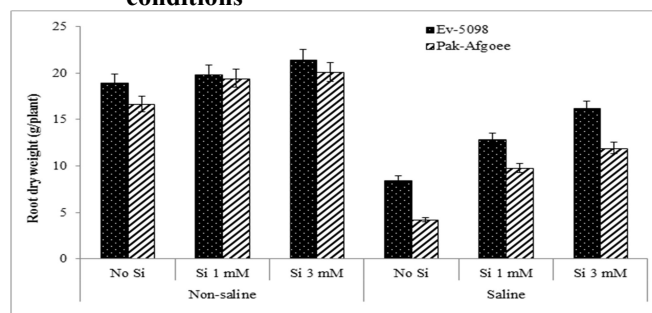


Figure 4. Effect of Si application on root dry weight of Maize genotypes under non- saline and saline conditions

The comparison of genotypes under Si application @ 1mM and 3mM showed that with 100 mM NaCl application, Ev-5098 had lower concentration of Na^+ as compared to Pak-afgoee which is salt sensitive genotype. Under 100 mM NaCl level of salinity, the minimum Na^+ concentration ($0.11 \text{ mmol g}^{-1} \text{ dwt}$) was found in the leaf of Ev-5098 with 3mM Si application in nutrient solution. The concentration of K^+ as against of Na^+ increase significantly at increasing level of Si application under salinity (Fig. 5 and 6) with potassium application. The highest K concentration in leaf of Ev-5098 ($0.14 \text{ mmol g}^{-1} \text{ dwt}$) was observed under 100 mM NaCl culture with Si application @ 3mM and it was statistically significant with Pak-afgoee ($0.08 \text{ mmol g}^{-1} \text{ dwt}$). It was predicted from Fig. 7 and 8 that the Zn concentration and Zn use efficiency improved under salinity (100 mM NaCl) with the application of silicon. The highest leaf Zn concentration was observed in leaf of Ev-5098 under saline environment with 3mM application of Si as compared Pak-afgoee. Similarly, the highest Zn efficiency was recorded in this genotype under saline condition while it was less as compared to non-saline conditions.

Zn and Fe use efficiency calculations: The Zn and Fe use efficiency was calculated by using the following equation:

$$\text{Zn use efficiency} = (\text{dry matter yield at Zn-deficient level} / \text{dry matter yield at Zn-sufficient level}) \times 100$$

$$\text{Fe use efficiency} = (\text{dry matter yield at Fe-deficient level} / \text{dry matter yield at Fe-sufficient level}) \times 100 \text{ (Graham, 1992).}$$

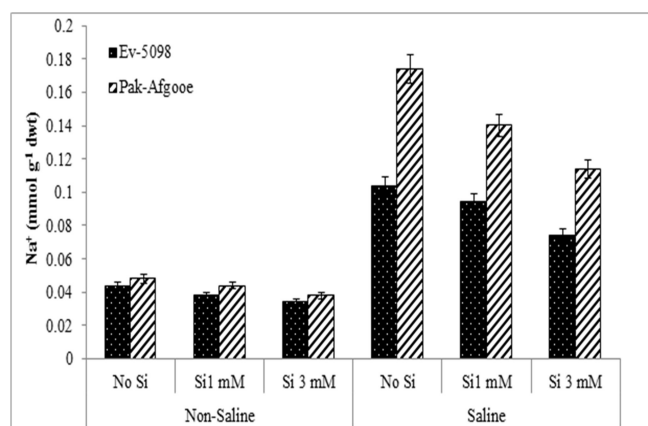


Figure 5. Effect of Si application on sodium concentration of Maize genotypes under non-saline and saline conditions

Similar trend was observed in Fe leaf concentration (Fig. 9) and Fe use efficiency (Fig-10). The genotype Ev-5098 contain $0.80 \text{ } \mu\text{mol g}^{-1} \text{ dwt}$ Fe in leaf with Si application @ 3 mM and 100 mM NaCl and it was statistically significant from Pak-afgoee. In case of Fe use efficiency, Ev-5098 was 66.6 % more efficient in Fe utilization as compared to Pak-afgoee under saline condition with Si @ 3mM.

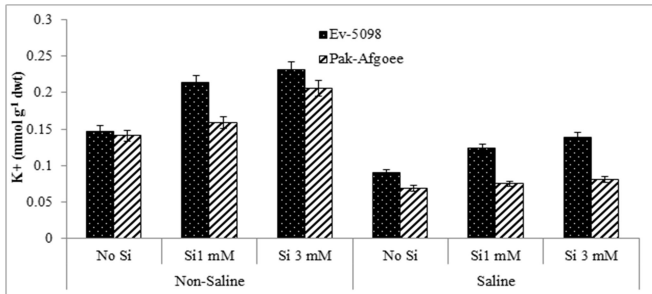


Figure 6. Effect of Si application on potassium concentration of Maize genotypes under non-saline and saline conditions

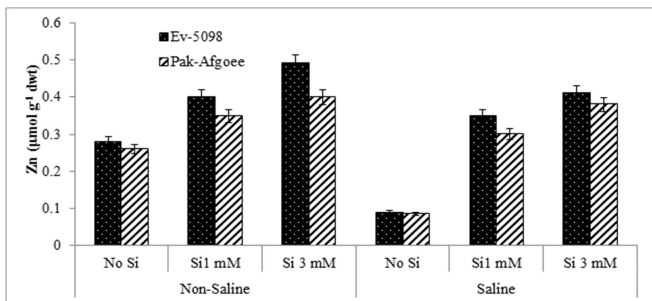


Figure 7. Effect of Si application on zinc concentration of Maize genotypes under non-saline and saline conditions

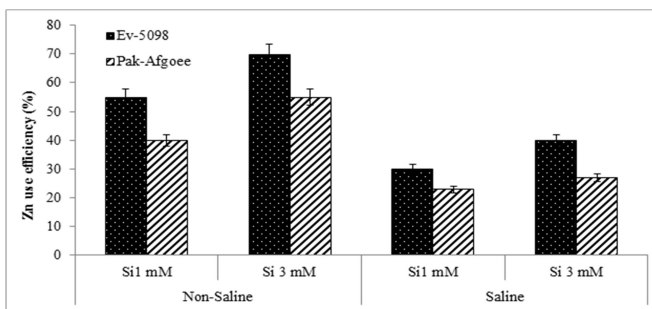


Figure 8. Effect of Si application on Zn use efficiency of Maize genotypes under non-saline and saline conditions

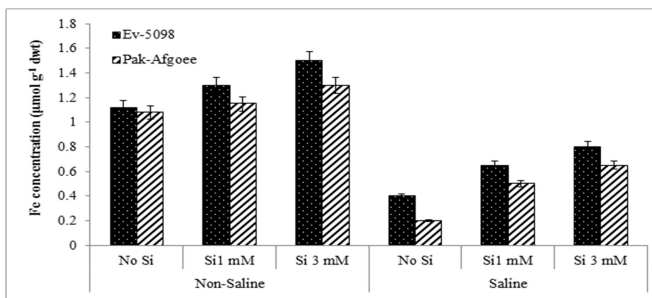


Figure 9. Effect of Si application on iron concentration of Maize genotypes under non-saline and saline conditions

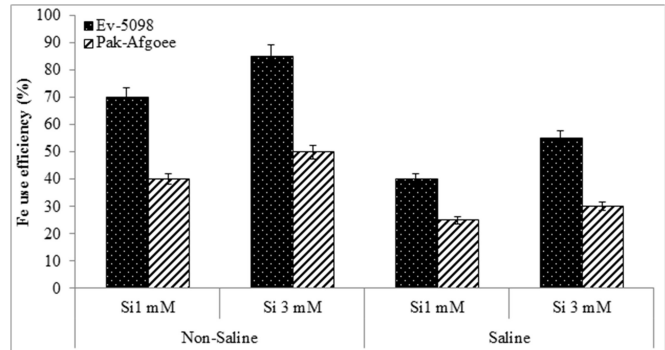


Figure 10. Effect of Si application on Fe use efficiency of Maize genotypes under non-saline and saline conditions

Chlorophyll Content (SPAD value): The data regarding chlorophyll content showed that the mean chlorophyll content of the maize genotypes has been decreased significantly under saline conditions while application of Silicon minimizes the negative effect of salinity (Fig-11). The individual as well as interactive effects of salinity, Silicon and genotypes were found significant. The comparison of genotypes under Si application @ 1mM and 3mM showed that with 100 mM NaCl application, Ev-5098 had higher chlorophyll content as compared to Pak-afgoee which is salt sensitive genotype. Under 100 mM NaCl level of salinity, the minimum chlorophyll content (32.4) was found in pak-afgoee with 0mM Si application in nutrient solution.

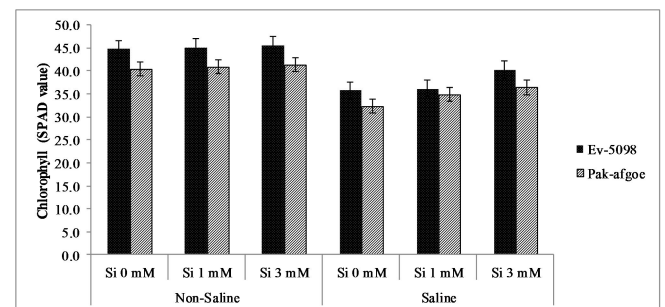


Figure 11. Effect of Si application on Chlorophyll Content (SPAD value) of Maize genotypes under non-saline and saline conditions

Photosynthetic Rate ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$): The data regarding photosynthesis rate shows that the mean photosynthesis rate of the maize genotypes has been decreased significantly under saline conditions while application of Silicon minimizes the negative effect of salinity (Fig-12). The individual as well as interactive effects of salinity, Silicon and genotypes were found significant. The comparison of genotypes under Si application @ 1mM and 3mM showed that with 100 mM NaCl application, Ev-5098 had higher

photosynthesis rate as compared to Pak-afgoee which is salt sensitive genotype. Under 100 mM NaCl level of salinity, the minimum photosynthesis rate ($13.5 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) was found in pak-afgoee with 0mM Si application in nutrient solution.

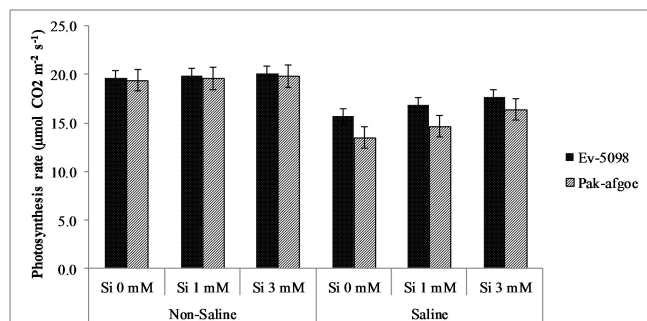


Figure 12. Effect of Si application on Photosynthesis Rate of Maize genotypes under non- saline and saline conditions

Transpiration Rate ($\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$): The data regarding transpiration rate shows that the mean transpiration rate of the maize genotypes has been decreased significantly under saline conditions while application of Silicon minimizes the negative effect of salinity (Fig-13). The individual as well as interactive effects of salinity, Silicon and genotypes were found significant. The comparison of genotypes under Si application @ 1mM and 3mM showed that with 100 mM NaCl application, Ev-5098 had higher transpiration rate as compared to Pak-afgoee which is salt sensitive genotype. Under 100 mM NaCl level of salinity, the minimum transpiration rate ($3.5 \text{ mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$) was found in pak-afgoee with 0mM Si application in nutrient solution.

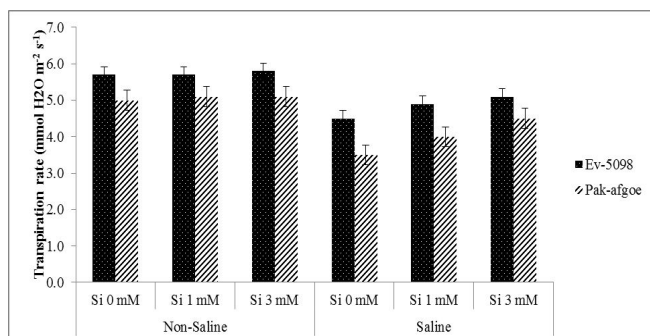


Figure 13. Effect of Si application on Transpiration Rate of Maize genotypes under non- saline and saline conditions

Stomatal conductance ($\text{mmol m}^{-2} \text{ s}^{-1}$): The data regarding stomatal conductance shows that the mean stomatal conductance of the maize genotypes has been decreased significantly under saline conditions while application of

Silicon minimizes the negative effect of salinity (Fig-14). The individual as well as interactive effects of salinity, Silicon and genotypes were found significant. The comparison of genotypes under Si application @ 1mM and 3mM showed that with 100 mM NaCl application, Ev-5098 had higher stomatal conductance as compared to Pak-afgoee which is salt sensitive genotype. Under 100 mM NaCl level of salinity, the minimum stomatal conductance ($153.7 \text{ mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$) was found in pak-afgoee with 0mM Si application in nutrient solution.

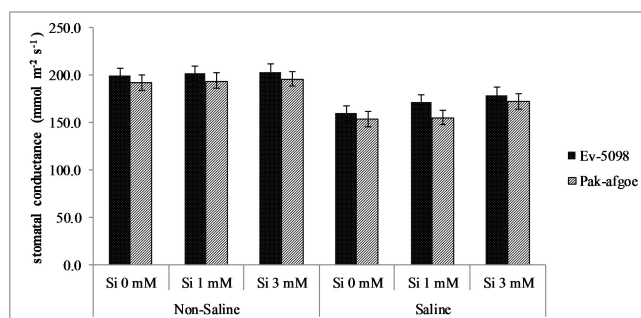


Figure 14. Effect of Si application on Stomatal conductance of Maize genotypes under non-saline and saline conditions

Water potential (-MPa): It was predicted from (Fig-15) that the mean water potential of the maize genotypes has been decreased significantly under saline conditions while application of Silicon minimizes the negative effect of salinity. The individual as well as interactive effects of salinity, Silicon and genotypes were found significant. The comparison of genotypes under Si application @ 1 mM and 3 mM showed that with 100 mM NaCl application, Ev-5098 had higher water potential as compared to Pak-afgoee which is salt sensitive genotype. Under 100 mM NaCl level of salinity, the minimum water potential (-0.80 MPa) was found in pak-afgoee with 0mM Si application in nutrient solution.

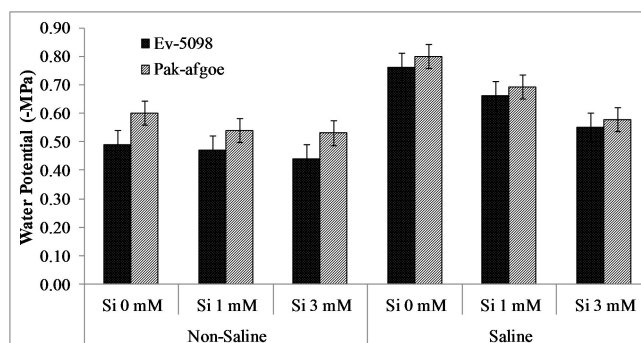


Figure 15. Effect of Si application on water potential of Maize genotypes under non- saline and saline conditions

DISCUSSION

One third of the world irrigated land is facing excessive salinity annually which ultimately affect crop production. Salinity is one of the major factors limiting plant growth and crop productivity by unbalancing cellular ions which results in ion toxicity and osmotic stress (Tester and Davenport, 2003). A significant growth reduction of the maize genotypes has been observed by NaCl salinity with and without application of Si application. Moreover, the toxic effect of salt stress was higher in the salt susceptible genotypes Pak-afgoee than in the salt resistant maize genotype EV-5098 and in most of the cases these two genotypes differed significantly. Some other researchers (Zhu *et al.*, 2004) also reported the character of Si against the adverse effect of salinity on a variety of crop plants such as wheat, rice, barley, tomato, cucumber and mesquite and concluded that Si plays a protective role against the salinity stress. Salinity decreases chlorophyll content and affects the photosynthetic electron transport thus inhibiting the PS-II activity as a result of salt toxicity in chloroplasts (Sudhir and Murthy, 2004). Salinity lowers the water potential and disturbs the ion distribution and plant adjust their water potential to more negative levels (Koyro, 2006; Suarez, 2006). Similar results were observed by Izzo *et al.* (1991) that salt stress lowered the water potential in maize leaves. However salt tolerant plants have ability to adjust their tissue water potential to a level that is lower than that of water potential of saline medium in which they are growing. Under salinity lower osmotic potential is most depressing effect in plants for nutrient and water uptake (Munns, 2002; Rengasamy *et al.*, 2006). Similar results were observed in rice (Sultana *et al.*, 1999), wheat (Sairam *et al.*, 2002), maize (Tuna *et al.*, 2008), and *glycine max* seedling (Chen and Yu, 2007). Liang *et al.* (2006) suggested that during NaCl stress, Si enhances the GSH content and maintains the optimal membrane fluidity and also plasma membrane H⁺-ATPase, thus reducing oxidative stress. Further, Tuna *et al.* (2008) reported that addition of Si reduced the rate of sodium transportation into roots and shoots under salt stress; however, at the same time shoot K and Ca concentrations were appreciably improved.

Conclusion: In the present study it was concluded that the salt sensitive genotype Pak-afgoee showed more negative value of water potential than salt tolerant genotype EV-5098. The leaf chlorophyll contents, net photosynthetic rate, stomatal conductance and transpiration rate have a strong negative correlation with leaf Na⁺ concentration and a strong positive correlation with leaf K⁺ concentration. More concentration of Fe, Zn and Si was observed in EV-5098 compared to Pak-afgoee. This study shows that silicon application under saline conditions increase the Fe and Zn

concentration which ultimately improves Fe and Zn use efficiency and growth of maize.

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