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Kentucky bluegrass (*Poa pratensis* L.) silicon-treated turfgrass tolerance to short- and long-term salinity condition

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Key words: chlorophyll content, proline content, salt stress, Si, turfgrass.

Abstract: The effects of short- and long-term salinity condition were investigated on silicon-treated and control plants of Kentucky bluegrass (KBG) (*Poa pratensis* L.) in a greenhouse study. Salt stress solely affected visual quality at ≥15 dS m^{-1} concentrations while Si application increased salt tolerance of KBG after 45 days. In long-term salinity stress, Si had no effect on salt tolerance of KBG at ≥15 dS m^{-1} concentration. Si increased morphological parameters including height and number of shoots, and physiological parameters including relative water content (RWC) and chlorophyll content of leaves. In addition, fresh and dry weights of roots and shoots in response to high salt concentrations declined, but showed an increase with Si treatment. Proline content and electrolyte leakage (EL) increased under high salinity levels. In response to the Si treatment, Na concentration in the shoots significantly decreased at the 5 dS m^{-1} salinity level. With increasing salinity levels, the concentration of K in roots and shoots decreased while the amount of K in both Si-treated roots and shoots reduced. Overall, Si alleviative effects were more pronounced in 45 days after turfgrasses being salinity treated.

1. Introduction

Kentucky bluegrass (*Poa pratensis* L.), a native to Europe, is the most commonly used cool-season turfgrass in the temperate and subarctic regions of North America, and it is also recognized for its ability to create a high-quality turf (Fry and Huang, 2004). Saline soils reduce growth due to osmotic and ion stresses (Marschner and Part, 1995; Munns, 2002). Salinity causes stress in plants in two ways, and plants respond in two distinct phases through time: a rapid response to the increase in external osmotic pressure, and a slower response due to the accumulation of Na^+ in leaves (Munns and Tester, 2008).

It is generally accepted that silicon can positively affect growth and health status of plants under biotic (Adatia and Besford, 1986; Ma, 2004) and abiotic (Barceló *et al.*, 1993; Ranganathan *et al.*, 2006) stresses. Acceptable results of silicon application against NaCl stress have been shown in rice (Matoh and Kairusmee, 1986; Yeo *et al.*, 1999), wheat (Ahmad *et al.*, 1992; Tuna *et al.*, 2008; Tahir *et al.*, 2010; Chen *et al.*, 2014), and barley (Liang *et al.*, 1996; Liang, 1999). Possible mechanisms for salt tolerance with the utilization of silicon have been proposed. These include accumulation of silicon in leaves resulting in reduced transpiration (Matoh and Kairusmee, 1986), turgor enhancement (Romero-Arandaet *al.*, 2006), formation of Na complexes in roots (Ahmad *et al.*, 1992), increased photosynthetic activity and protection of plasmatic membranes and chloroplast ultrastructures (Liang *et al.*, 1996; Liang, 1998; Shu and Liu, 2001), protection of plant tissues from free radicals through increasing the activity of antioxidative enzymes (Liang, 1999; Liang *et al.*, 2003; Zhu *et al.*, 2004), and alleviation of specific ionic effects (Rafiq, 1990) by reducing Na uptake (Liang, 1999; Epstein, 2001; Gong *et al.*, 2003).

Gong *et al.* (2003) also observed improved water economy and dry matter yield of plants with Si application. Silicon application is reported to enhance leaf water potential in wheat under drought stress (Liang, 1999). The authors suggested that a double layer, comprised of silica and cuticle on leaf epidermal tissue, is responsible for this higher water potential. Silicon application enhances water use efficiency, heat/salt tolerance, and resistance to pathogens and

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heavy metals (Yeo *et al.*, 1999; Zwieniecki *et al.*, 2001; Gao *et al.*, 2004; Liang *et al.*, 2005 a, b; Wang *et al.*, 2005; Guo *et al.*, 2007; Liang *et al.*, 2007). It has been highly recommended to use Si in turfgrass management (Datnoff and Rutherford, 2003; Datnoff and Rutherfood, 2004). In another study on *Poa pratensis* L. 'Baron', Chai *et al.* (2010) reported that Si application under salinity condition raised the transfer of K^+ from roots to shoots, but inhibited the absorption and transfer of Na^+, which may contribute to better turf quality and growth with Si treatment under saline conditions.

Chen *et al.* (2014) also found that Si can enhance plant salt tolerance by alleviating the salt-induced osmotic stress. Mateos-Naranjo *et al.* (2013) showed that in wheat, the alleviative effects were more pronounced in the osmotic stress phase than the ion toxicity phase. These results clearly showed that Si can enhance plant salt tolerance by alleviating the salt-induced osmotic stress.

The purpose of the present study was to evaluate the short- and long-term effects of salinity on growth and physiological parameters of Kentucky bluegrass with and without silicon treatment.

2. Materials and Methods

Plant materials and growth conditions

The experiment was conducted for a period of 90 days in the greenhouse of the Department of Horticultural Science, College of Agriculture, Shiraz University, Shiraz, Iran. Plastic pots (20 cm in diameter and 10 cm in depth) were filled with 1.3 kg of a mixture of 1:1 sand and perlite, then seeds were cultured. The field capacity and permanent wilting point for the potting mixture were 10% and 4%, respectively. During the germination stage, turfgrasses were irrigated daily with 150 ml deionized water until plants were adequately established. Then, irrigation was carried out every two days with 200 ml deionized water. Before treatments began, the turfgrass was clipped to 5-7 cm every two weeks. The nutrient solution of a commercial whole fertilizer (Cristalone) was used weekly with a concentration of 0.1%. The EC of the nutrient solution was 1 dS m^{-1}. Mean relative humidity, daily temperature and light condition of the greenhouse were categorized as 45±5%, 24±4°C and 29 Wm^{-2} (16/8 h day/night), respectively.

Treatments

Silicon was applied in the form of potassium silicate (K_2SiO_3) at a concentration of 1 mM as foliar application weekly from 1 September to 1 December 2009. Salinity treatment began two weeks after the silicate treatment. Different salt concentrations were prepared by adding 1 NaCl: 1 $CaCl_2$ (w/w) to deionized water to obtain desired EC values; saline water of 5, 10, 15 and 20 dS m^{-1} along with deionized water as the control was applied (200 ml per pot) every two days. To prevent salinity shock, salinity levels were increased stepwise by 5 dS m^{-1}.

Measurements

Physiological parameters were measured twice: at 45 and 90 days following commencement of salinity treatments. Chlorophyll content, Relative water content (RWC), electrolyte leakage and proline content were measured.

Chlorophyll content was measured according to the method of Saini *et al.* (2001). Half a gram of fresh leaf material, taken from the youngest fully expanded leaf, was extracted with 80% acetone and read using a spectrophotometer at 645 and 663 nm wavelengths. Chlorophyll content was calculated using the following formula:

$$mgChl/g\ f.w. = [(20.2(OD\ 645\ nm) + (8.02(OD663nm))]*V/(f.w.*1000)$$

where V is the final solution volume in ml and f.w. is tissue fresh weight in mg.

Relative water content was measured using the methods of Nepomuceno *et al.* (1998) and Sairam *et al.* (2002). The value of RWC was determined by the following equation:

$$RWC\ (\%) = [(f.w.-d.w.)/(t.w.-d.w.)]\times100$$

where f.w. is the fresh weight, d.w. is the dry weight and t.w. is the turgid weight.

Electrolyte leakage was determined according to the methods described by Saadalla *et al.* (1990).

Samples of 0.1 g of fresh leaves were weighed and washed three to four times with deionized water and immersed in a test tube containing 15 ml deionized water, then maintained at 25°C for 24 h. The tubes were then shaken for 15 min and the conductivity of the solution was measured (EC1) using an electrical conductivity meter (Metrohm 644, Swiss). The test tubes were then placed in an autoclave at 0.1 MPa for 10 min to kill the plant tissue and release all of the electrolytes. The tubes were cooled to 25°C, shaken, and their solution conductance measured again (EC2). The electrolyte leakage was calculated as EC1/EC2 and expressed as percent.

Proline content was calculated according to the

Bates *et al.* (1973) method. A half gram of fresh leaves was homogenized with 10 ml of 3% aqueous sulfosalicylic acid and filtered through Whatmans no. 2 filter paper. Two ml of filtrate was mixed with 2 ml of acid-ninhydrin and 2 ml of glacial acetic acid in a test tube. The mixture was placed in a water bath at 100°C for 1 h. The reaction mixture was extracted with 4 ml toluene, and the absorbance was measured at 520 nm with a spectrometer (UV-120-20, Japan). Standard curves of proline were used for the calculation of proline amount in the samples.

For fresh and dry weight determination of shoot and root systems and further chemical analysis, leaves and roots were washed three to four rinses in distilled water and then dried at 70°C for 48 h. The dried leaves and roots were ground to powder using an electric mill (AR 10, Molinex, China) and subsequently stored in polyethylene bottles at room temperature. One gram of leaf sample was ashed in a furnace at 550°C for 5 h. The ash was then dissolved in 10 ml 2N HCl and diluted to the volume of 100 ml with distilled water. Potassium and sodium contents were determined using a flame photometer (PFP7, Jenway, England) (Champan and Pratt, 1982).

Statistical analysis

The experiment was conducted in a complete randomized design with three replications. Data were analyzed using MSTAT-C software. Means were compared using the least significant difference (LSD) test at (P<0.05) level.

3. Results

The turfs treated with silicon, even at high concentration of salinity, maintained their visual quality and turf performance while those without silicon at 20 dSm^{-1} concentration died after 45 days. During long-term salinity exposure, either with or without Si, Kentucky bluegrass lost turf performance at 15 and 20 dS m^{-1} concentrations (data not shown). Salt stress reduced chlorophyll content during short-term salinity. Silicon treatment increased chlorophyll content extensively both under non saline irrigation and various concentrations of salty solutions (Fig. 1).

Under different salinity exposures, RWC increased through Si treatment. Results were significantly different in the presence and absence of silicon under non saline conditions. Significant differences were observed between salinity control groups, treated with Si of otherwise (Fig. 2).

As shown in figure 3, increased salinity resulted in remarkably enhanced electrolyte leakage. Silicon reduced electrolyte leakage after 45 days of salinity. In lower concentrations of salinity, silicon reduced

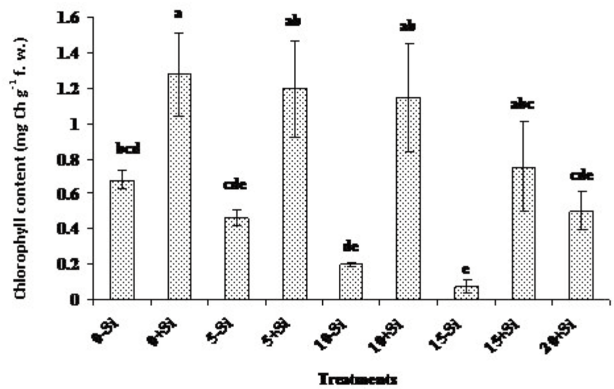

Fig. 1 - Effects of different salinity and Si levels on chlorophyll content of *Poa pratensis* after 45 days. 0: control, 5, 10, 15 and 20 dS m^{-1} as salinity levels; Si: K_2SiO_3 1 mM. Data are mean ± SE at P<0.05 using LSD test.

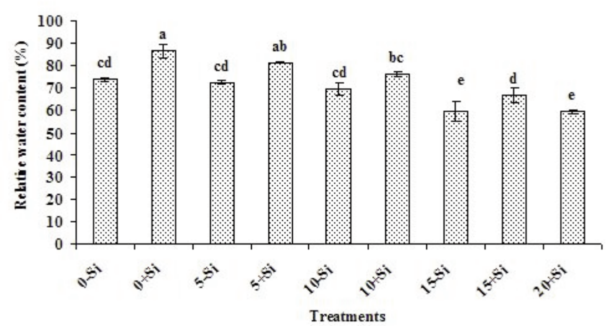

Fig. 2 - Effects of different salinity and Si levels on relative water content in of *Poa pratensis* after 45 days. 0: control, 5, 10, 15 and 20 dS m^{-1} as salinity levels; Si: K_2SiO_3 1mM. Data are mean ± SE. at P<0.05 using LSD test.

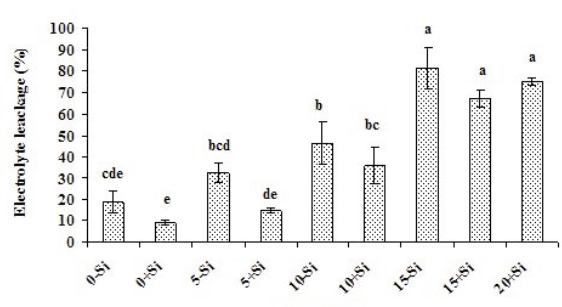

Fig. 3 - Effects of different salinity and Si levels on electrolyte leakage of *Poa pratensis* after 45 days. 0: control, 5, 10, 15 and 20 dS m^{-1} as salinity levels; Si: K_2SiO_3 1 mM. Data are mean ± SE. at P<0.05 using LSD test.

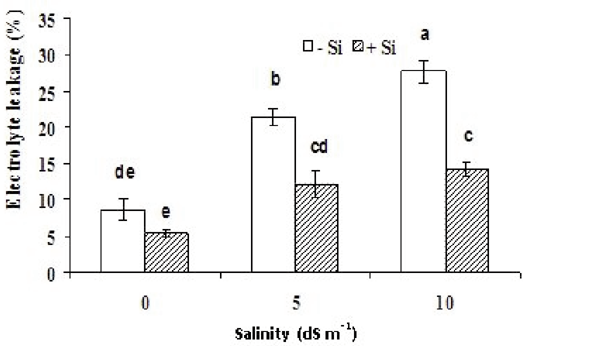

Fig. 4 - Interaction effects of different salinity and Si levels on electrolyte leakage of *P. pratensis* after 90 days. Data are mean ± SE at P ≤0.05 using LSD test.

electrolyte leakage even after 90 days (Fig. 4).

Proline concentration increased dramatically after 45 and 90 days of salt stress, although plants with Si application had less proline content (Figs. 5 and 6).

Higher concentrations of saline irrigation showed more reduction in shoot number of *P. pratensis*. Silicon markedly increased shoot numbers at lower concentrations (Table 1). A dramatic reduction in visual quality based on shoot density and percentage of green leaf canopy area (GLCA) was observed after 45 days when *P. pratensis* was cultured at different

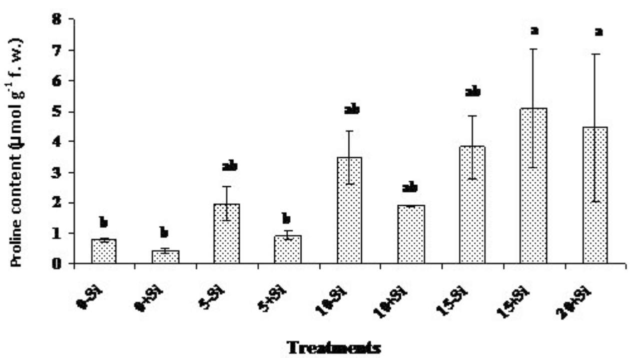

Fig. 5 - Effects of different salinity and Si levels on proline content of *Poa pratensis* after 45 days. 0: control, 5, 10, 15 and 20 dS m^{-1} as salinity levels; Si: K_2SiO_3 1 mM. Data are mean ± SE. at P<0.05 using LSD test.

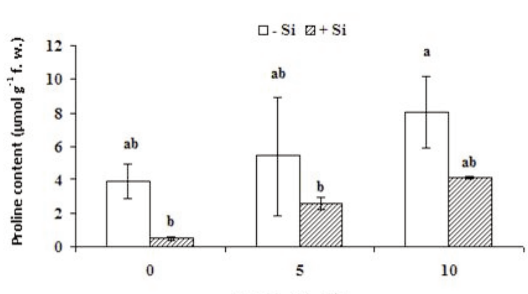

Fig. 6 - Interaction effects of different salinity and Si levels on proline content of *P. pratensis* after 90 days. Data are mean ± SE at P ≤0.05 using LSD test.

Table 1 - Interaction effects of different salinity and Si levels on growth parameters, shoot and root Na and K concentrations of *Poa pratensis*

		Treatments		
		Salinity (dS m^{-1})		
Indicator		0	5	10
Shoot length (cm)	-Si	24.57 ab	20.93 bc	17.37 c
	+Si	29.27 a	20.53 bc	16.4 c
Shoot number in pot	-Si	337 ab	121.3 c	62.67 c
	+Si	378.7 a	340.7 ab	258.7 b
Shoot fresh weight (g)	-Si	22.57 ab	10.28 cd	3.413 d
	+Si	29.43 a	16.90 bc	8.313 cd
Root fresh weight (g)	-Si	46.67 ab	23.57 cd	20.61 d
	+Si	50.20 a	34.96 bc	18.58 d
Shoot dry weight (g)	-Si	6.116 ab	3.506 bc	0.947 c
	+Si	9.791 a	6.925 ab	2.033 c
Root dry weight (g)	-Si	14.28 ab	6.372 c	6.109 c
	+Si	21.28 a	12.45 bc	5.744 c
Shoot Na concentration (g)	-Si	360.6 c	948.0 a	826.2 ab
	+Si	234.3 c	562.1 bc	554.6 bc
Root Na concentration (g)	-Si	40.22 cd	102 ab	118.3 a
	+Si	29.06 d	71.48 bc	81.15 ab
Shoot K concentration (g)	-Si	542.2 ab	347.2 bc	175.6 c
	+Si	861.4 a	374.3 bc	216.3 c
Root K concentration (g)	-Si	12.16 c	3.966 d	2.242 d
	+Si	26.15 a	18.37 b	13.41 c

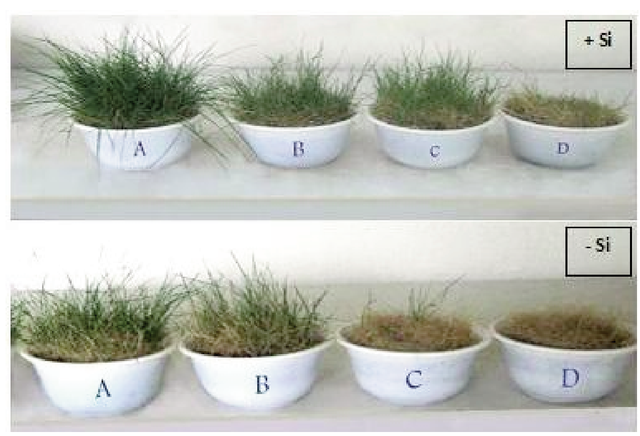

Fig. 7 - Comparison of different concentrations of salts (A: 5 dS m^{-1}, B: 10 dS m^{-1}, C: 15 and D: 20 dS m^{-1} with Si (+Si) and without Si (-Si) in *P. pratensis* 45 days after beginning the treatments.

levels of salinity (Fig. 7).

Shoot fresh and dry weight of *P. pratensis* showed the most prominent decrease at 5 dS m^{-1} salt concentration (Table 1). Also, a significant decline in root fresh and dry weight was observed at 5 dS m^{-1} (Table 1). Si application partially enhanced shoot fresh and dry weight. In addition, Si had a greater impact on root fresh weight with non-saline irrigation (Table 1). In the leaves and roots, Na^+ content significantly increased at low concentrations of salty irrigations after 90 days. In contrast, shoot K^+ content was significantly less when salinity level reached 10 dS m^{-1}. Si treatment increased shoot K^+ content in comparison to saline irrigations without Si application. Root K^+ content reduced markedly at 5 dS m^{-1}, compared to the control. Turfs treated with Si had a higher concentration of K^+ in the roots (Table 1).

4. Discussion and Conclusions

Kentucky bluegrass (KBG) is generally considered to be a salt sensitive turf. In our research, KBG had no tolerance at periodically extended concentrations higher than 15 dS m^{-1} salinity, regardless of silicon application; the salinity tolerance threshold of KBG resulted to be 10dS m^{-1}. Silicon could increase salinity tolerance at higher concentrations.

The silicon remedy was more pronounced during short-term saline conditions, findings that are consistent with previous reports. Silicon could increase the amount of chlorophyll and photosynthesis and consequently, growth. Si protects plasmatic membranes and chloroplast ultrastructures (Liang *et al.*, 1996; Liang, 1998; Shu and Liu, 2001), stimulates H^+-ATPase activity, and increases K^+ in shoots (Liang *et al.*, 1996; Liang *et al.*, 2003; Liang *et al.*, 2005 a). Furthermore, it improves the activity of antioxidant enzymes, reducing the damage of reactive oxygen species (ROS) (Liang, 1999; Liang *et al.*, 2003; Zhu *et al.*, 2004) and reduces Na^+ root uptake, alleviating specific ion effects (Epstein, 2001; Gong *et al.*, 2003; Liang *et al.*, 2003).

It has been reported that the accumulation of Si in plants enhances the strength and rigidity of the tissues (Ma and Yamaji, 2006; Neethirajan *et al.*, 2009). An increased Si supply improves the structural integrity of crops and may also improve plant tolerance to disease, drought, and metal toxicities (Yeo *et al.*, 1999; Richmond and Sussman, 2003; Ma *et al.*, 2004). These findings are in agreement with our study. Some researchers hypothesized that Si deposition in the cell wall of root endoderm may contribute to the maintenance of the apoplastic barrier and thereby improve plant tolerance to disease and drought stress (Lux *et al.*, 2002; Lux *et al.*, 2003; Hattori *et al.*, 2005).

Electrolyte leakage was influenced more in the short-term salinity stress than long-term salinity stress. It appears that plant adaptability to lower salinity levels in the short term increases resistance to salt. As shown in figures 5 and 6, a significant increase in proline content was observed at 15 dS m^{-1}. However, silicon only slightly affected proline levels compared to the turfgrasses treated with saline and non-saline waters. Proline often accumulated in grasses under salinity stress, however this amount of content was insufficient for osmotic adjustment in grasses (Marcum, 2002).

Foliar application of Si increased the unsaturated fatty acid ratios [(18:2+18:3)/18:1] in glycolipids and phospholipids and also proliferated the amount of membrane lipids in strawberries (Wang and Galletta, 1998). Agarie *et al.* (1998) noted that Si increased membrane stability of rice under drought and heat stresses, which prevented the structural and functional deterioration of cell membranes. In concordance, it appears that Si plays an important role in maintaining the integrity, stability and function of cell membranes in Kentucky bluegrass under salt stress.

Ashraf and Foolad (2007) reported an increase in the amount of proline primarily in cytosols under salinity stress. They found that plant tolerance to salinity stress and proline accumulation are positively related. However, the relationship is not universal and might be cultivar dependent.

Moreover, Bartels and Sunkar (2005) and Ashraf and Foolad (2007) reported other possible roles attributable to proline besides osmotic adjustment in stressed plants, such as acting as a hydroxyl scavenger, the stabilization of membrane and protein structure, serving as a sink for carbon and nitrogen during stress recovery, and the buffering of cellular redox potential under stressful conditions.

As shown in figure 2 RWC reduced significantly under higher salinity levels in short-term salt stress. A probable explanation for an increase in RWC under Si application may be the prevention of transpiration.

Marcum and Murdoch (1990) reported that shoot water content of *Zoysia matrella* (L.) Merrill, *Z. japonica* L., *Paspalum vaginatum* Swarts and *C. dactylon* decreased during a one month salinity stress in solution culture, and suggested that osmotic adjustment was not achieved exclusively by solute accumulation.

Si is known to decrease Na uptake (2004). The present results clearly show that Na uptake could be reduced through Si treatment. Similar results have been achieved in investigations by Epstein (2001), Liang *et al.* (2003), Gong *et al.* (2003, Chai *et al.* (2010), and Bae *et al*. (2012).

Shoot growth and leaf firing decreased as salinity levels increased (Fig. 7 and Table 1). Horst and Taylor (1983) stated that growth declines to 50% when the concentration of salt reaches 11 dS m^{-1} in 44 cultivars of Kentucky bluegrass, which was approximately similar to the value obtained in this study. An adverse result was reported by Alshammary *et al.* (2004) who stated that shoot growth decreased by 50% at 5.5 dS m^{-1} concentration of salinity (a mixture of NaCl and $CaCl_2$) in Kentucky bluegrass.

Under high salinity levels, cell expansion could be reduced by accumulation of salts in cell walls which would effectively reduce cell turgor and consequently retard growth (Oertli, 1968; Flower and Yeo, 1986). A decrease in growth at higher sodium concentrations due to a decrease in the uptake of K^+ and Ca^{2+} has also been reported (Sairam and Tyagi, 2004).

Si increased chlorophyll content, RWC, and visual quality under non saline water and low concentrations of salinity in both short- and long-term exposures. Proline content and electrolyte leakage increased in response to increasing salinity levels. K^+ concentration in shoots and roots increased as a result of Si application. Shoot number and shoot length dramatically decreased under higher salinity levels while Si application increased shoot number and shoot length under low concentration of salt stress. In general, silicate fertilizer is recommended for turfgrass management against environmental stresses such as salinity, high or low temperatures, drought and heavy metals.

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