Journal of Ornamental Plants
Available online on: www.jornamental.com
ISSN (Print): 2251-6433 ISSN (Online): 2251-6441

Effect of Silicon on Growth and Ornamental Traits of Salt-stressed Calendula (*Calendula officinalis* L.)

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Received: 17 December 2012 Accepted: 14 October 2013
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Abstract

A pot experiment was carried out to determine the effect of foliage spraying of silicon (Si) on growth and ornamental characteristics of calendula grown under salt stress and greenhouse conditions. A factorial experiment based on completely randomized design was conducted with 3 levels of Si (0, 50 and 100 mg/l) and 3 levels of NaCl (0, 100 and 200 mM) with 4 replications. At flowering stage, Si was applied with spraying two times in two week intervals. NaCl was also applied as drench (200 ml per pot) in two days interval. The results showed that salinity decreased the growth, SPAD values, flower number per plant and flower diameter. However, foliar applications of Si resulted in greater root, shoot and total dry weight, plant height and leaf area of calendula plants under salt stress. The highest SPAD values were obtained under 100 mg/l Si application in all NaCl treatments. Salinity decreased number of flower per plant and flower diameter as ornamental characteristics; however Si increased them under salinity stress. Plants treated with 100 mg/l Si had the highest flower diameter at 100 mM of NaCl. Electrolyte leakage increased by salinity, however foliar application of Si significantly reduced electrolyte leakage under salt stress. These results suggest that the negative effects of salinity on the growth and ornamental characteristics of calendula plants can ameliorate by foliar application of Si treatments.

Keywords: Electrolyte leakage, Foliar application, Landscape, Salinity, SPAD value.

INTRODUCTION

One of the most important environmental factors limiting plant growth and productivity is salinity (Kaya *et al.,* 2003). In most arid and semiarid areas, this problem is accentuated by competition for high quality water among agriculture, industry and landscape users has promoted the use of alternative water sources for irrigation. Thus, marginal quality water, somewhat saline, will become important in these areas (Chartzoulakis *et al.*, 2002) and could be used for the irrigation of ornamental plants (Carter *et al*., 2005). However, the use of low quality water for irrigation affects plants in different ways, depending on the degree of salt tolerance of the species (Alarcon *et al.,*1994) and even within a given species (Sanchez-Blanco *et al.,* 2003). Salt stress can affect plant survival, biomass, plant height and plant morphology and affect the capacity of a plant to collect water and nutrients (Parida and Das, 2005). Salinity can cause hyper ionic and hyper osmotic effects on plants leading to membrane disorganization, increase in reactive oxygen species (ROS) levels and metabolic toxicity (Jaleel *et al.*, 2007a). The great effect of salinity is the inhibition of crop growth by the reduced hormone delivery from root to leaves (Jaleel *et al.*, 2007b).

Calendula, mostly known as the pot marigold, is planted widely in gardens and landscapes. It is popular for the lush color and aromatic scent. It grows in sun or partial shade and is easy to grow requiring little cultivation (Dole and Wilkins, 2004).

Silicon (Si) is the second most abundant element on the surface of the earth, yet its role in plant biology has been poorly understood. Silicon has not been considered an essential element for the growth of higher plants; however, soluble Si has enhanced the growth, development and yield of several plant species including rice (*Oryzae sativa* L.), sugarcane (*Saccharum officinarum* L.) and most other cereals and several dicotyledons (Jones and Handreck, 1967; Elawad and Green, 1979; Takahashi *et al*., 1990; Belanger *et al.,* 1995; Savant *et al.*, 1999), especially under biotic and abiotic stress conditions (Epstien, 1994). Silicon concentration in the soil solution is controlled by silicate minerals and ranges from 0.01 to 1.99 mM (Karathanasis, 2002). Silicon plays a significant role in imparting biotic and abiotic stress resistance and enhancing crop productivity (Okuda and Takahashi, 1965; Ma *et al.*, 1989; Epstein, 1994; Liang *et al.*, 1994). Improvement of salt tolerance has been reported by addition of Si in wheat (Ahmad *et al.*, 1992), barley (Liang *et al.,* 2003), rice (Yeo *et al.,* 1999), maize (Wang *et al.*, 2004), cucumber (Zhu *et al.*, 2004), rose (Savvas *et al.*, 2007) and zinnia (*Zinnia elegans*) (Kamenidou *et al.*, 2009). Savvas *et al*. (2002) reported that quality and peduncle stem thicknessin gerbera flower increased when potassium silicate was included in the hydroponic nutrient solution. Hwang *et al*. (2005) also reported that applications of potassium silicate improved the growth and quality of cut miniature rose 'Pinocchio' in the rock wool culture system.

Water deficiency is a serious problem in arid and semi-arid regions of Iran that characterized by little rainfall, high solar radiation and high temperatures in the summer. In recent years, the normal seasonal droughts that have occurred in Iran have caused local and state government to enact water conservation ordinances. Urbanization and increases in population, however, are seriously threatening sustainable natural resources. At present, non-renewable groundwater resources are being depleted to an alarming extent. As high-quality water supply becomes limited, the use of saline water with high salt levels for landscape irrigation is being encouraged. While crop tolerance to salinity has been given considerable attention; fewer studies have dealt specifically with ornamental plants. Therefore, the aim of this research was to investigate the effects of Si on growth and quality flower of calendula grown under normal and salinity stress conditions.

MATERIALS AND METHODS

Plant material and treatments application

For this experiment, the seeds of *Calendula officinalis* L. 'Nana Bon Bon Naranja' were selected and germinated in cocopeat in plastic germination trays under greenhouse conditions at

Ferdowsi University of Mashhad (36°17'44" N and 59°36'42" E), Iran. Uniform size seedlings (40-days old) were transplanted to plastic pots (15 cm top diameter) and filled with mixture of loam soil:sand:compost (1:1:1,v:v). The plants were grown in a naturally illuminated greenhouse with night/day set temperatures of 18/24 °C. A factorial experiment based on completely randomized design was conducted with 3 levels of Si (0, 50 and 100 mg/l) and 3 levels of NaCl (0, 100 and 200 mM) with 4 replications. Potassium silicate (K_2SiO_3) was used for silicon treatments. Thirty days after transplanting, Si was applied on the foliage of calendula plants with a hand sprayer. The volume of the spray was 30 ml per pot. NaCl was also applied as drench (200 ml per pot) in two days interval. A control group of plants was grown without NaCl and sprayed with deionized water. All plants were harvested 60 days after planting (30 days after treatments) and separated into leaves, stem, and root.

Measurements and data collection

Dry weights of separated roots and shoots were recorded on four randomly selected plants per treatment. Total leaf area was determined with a Delta-T Image Analysis System (Delta-T, LTD, Cambridge, UK). Plant height, flower diameter and number of flower per plant were also measured. Electrolyte leakage which is used to assess membrane permeability was determined according to Lutts *et al.* (1996). Leaf greenness or chlorophyll reading values (measured as the optical density, SPAD value) was recorded at the end of the experiment on three leaves per plant at similar middle positions of shoots for all plants in each treatment using a portable SPAD chlorophyll meter (SPAD 502, Minolta, Japan).

Statistical analysis

Analysis of variance (ANOVA) for all the variables was carried out using the JMP8 software. Treatment means were compared using the protected Least Significant Difference (LSD) test at $p<0.05$ level.

RESULTS

Plant growth

Shoot, root and total dry weight were recorded as influenced by different levels of Si and NaCl. As shown in Fig. 1, shoot, root and total dry weight of calendula plants were lower at salt stress treatment as compared to non-saline conditions. However, foliar application of Si increased dry matter accumulation in all parts of calendula plants under salt stress. Application of Si (100 mg/l) under salt stress (100 mM NaCl) gave the higher values for these parameters than the other treatments. It indicated that foliar application of Si alleviated the growth inhibition induced by added NaCl. Exogenous application of Si also enhanced shoot, root and total plant dry weight under no salt stress (Fig. 1).

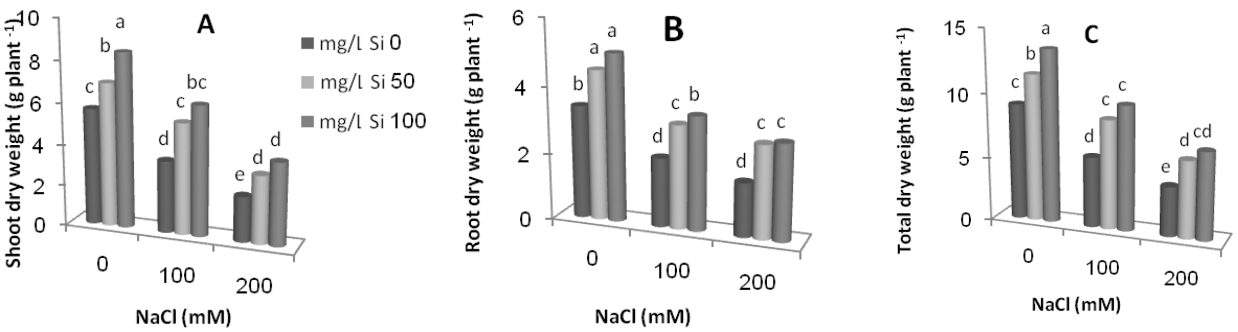

Fig. 1. The dry weight of shoot (A), root (B) and total plant (C) of calendula in response to foliar Si applications under salt stress. Different letters on top of bars indicate significantly differences according to LSD test ($p < 0.05$) at each salt level. Vertical bars indicate the mean ± SE.

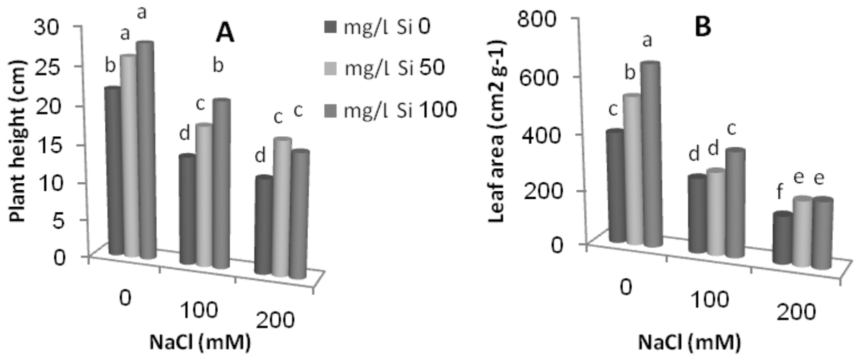

Fig. 2. Plant height (A) and leaf area (B) of calendula plant in response to foliar Si applications under salt stress. Different letters on top of bars indicate significantly differences according to LSD test ($p < 0.05$) at each salt level. Vertical bars indicate the mean ± SE.

Salt stress significantly decreased plant height; however, foliar application of Si improved plant height under salt stress (Fig. 2A). Treatment 100 mg/l Si + 100 mM NaCl had the highest plant height as compared to the other treatments under salt stress. Foliar application of Si (100 mg/l) increased plant height by 27% as compared to control in 100 mM NaCl (Fig. 2A).

Salt stress decreased leaf area as compared to the non-saline conditions. NaCl (200 mM) decreased leaf area by 58%, but the reduction was 41% when 100 mg/l Si treatment was applied to NaCl-treated plants, as compared to control. Under non-saline conditions, foliar application of Si (100 mg/l) significantly increased leaf area (1.6 fold) as compared to control (Fig. 2B).

Ornamental characteristics

Salt treatment (200 mM) significantly decreased number of flower per plant by 70% as compared to control, but the reduction was only 40% when Si (100 mg/l) was applied to NaCl-treated plants. Under non-salt stress condition, Si increased flower number by 35% as compared to control (Fig. 3A).

Flower diameter of calendula plants decreased dramatically with the increasing NaCl concentration. All Si treatments increased the flower diameter compared to non-treated plants both in absence and presence of salinity. Plants treated with 100 mg/l Si had the highest flower diameter at 100 mM of NaCl (Fig. 3B).

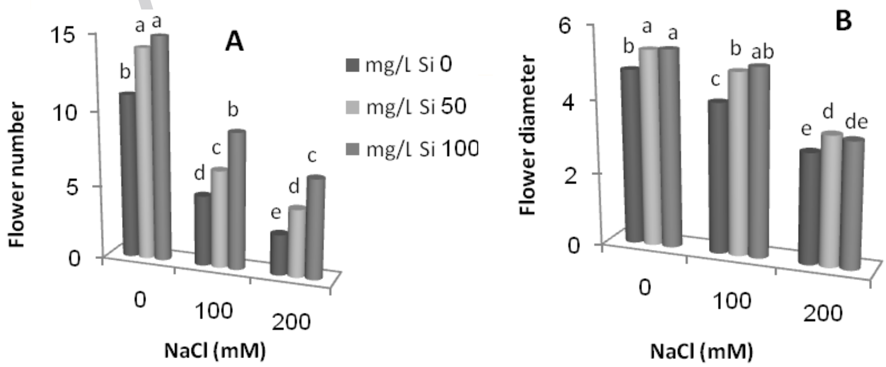

Fig. 3. Flower number per plant (A) and flower diameter (cm) (B) of calendula plant in response to foliar Si applications under salt stress. Differentletters on top of bars indicate significantly differences according to LSD test ($p < 0.05$) at each salt level. Vertical bars indicate the mean ± SE.

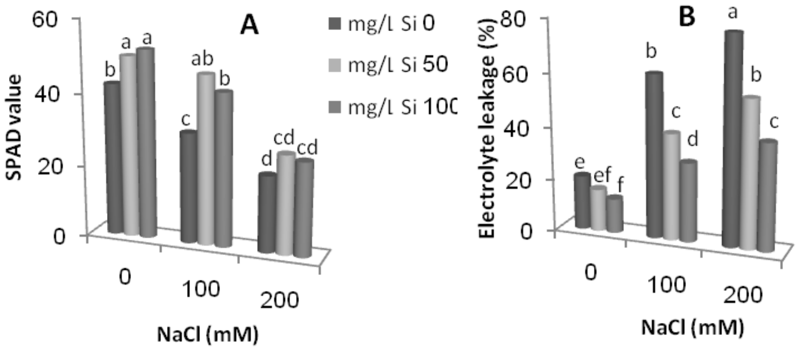

Fig. 4. SPAD value (A) and electrolyte leakage (B) of calendula plant in response to foliar Si applications under salt stress. Differentletters on top of bars indicate significantly differences according to LSD test ($p < 0.05$) at each salt level. Vertical bars indicate the mean ± SE.

SPAD valueand electrolyte leakage

SPAD values were significantly decreased with the increasing salinity stress. However, foliar Si applications were caused to the elevated SPAD values. Under saline conditions, the highest SPAD values were obtained from 50 mg/l Si application in all NaCl treatments (Fig. 4A).

Electrolyte leakage increased by salt treatment; however, Si significantly decreased electrolyte leakage under salt stress. The lowest electrolyte leakage was obtained by 100 mg/l Si in 100 mM NaCl concentration under salt stress condition. Under no salt stress, foliar application of Si only slightly decreased electrolyte leakage compared to control (Fig. 4B).

DISCUSSION

Under saline conditions, plant growth of calendula decreased significantly. Growth reduction under saline stress has been reported in various plants by many researchers (Senaratna *et al.,* 2000; Alpaslan and Gunes, 2001; Kaya *et al*., 2003; Sivritepe *et al*., 2003). As stated by Munns (2002), suppression of plant growth under saline conditions may either be due to decreased availability of water or to the toxicity of sodium chloride. Si treatments alleviated the deleterious effects of salinity on plant growth. Similar results were reported in rice (Matoh *et al*., 1986; Yeo *et al.*, 1999), maize (Wang *et al*., 2004), cucumber (Zhu *et al*., 2004), rose (Savvas *et al*., 2007) and zinnia (*Zinnia elegans*) (Kamenidou *et al*., 2009) who observed exogenous Si treatments ameliorated the negative effects of salt stress on plant growth. It has been observed that GA1 and it is precursor GA20 enhanced with N and Si application in rice cultivars (Hwang *et al*., 2008). Gibberellins affect cell enlargement and division which leads to internode elongation in stems and increases stem height. Under saline growth conditions, it has been reported that the benefits of Si are due to the reduction of Na content in the shoots of rice, P. juliflora, and barley (Matoh *et al*., 1986; Bradbury and Ahmad, 1990; Yeo *et al.*, 1999; Liang *et al.*, 2003), enhanced K uptake of barley (Liang *et al.*, 1996) and improved photosynthesis rate in barley and tomato (Liang, 1998; Al-Aghabary *et al.,* 2004). It has been also reported that Si increases the plant growth and yield of cucumber (Miyake and Takahashi, 1983; Voogt and Kreuzer, 1989).

Si increases rigidity of the mature leaves, which have a rougher texture and are held more horizontally, delays leaf senescence and increases chlorophyll content and ribulose, 1-5- bisphosphate carboxylase activity (Adatia and Besford, 1986). Moreover the induction of antioxidant enzymes and their protective role of membranes caused increasing the tolerance of plant to damages (Liang, 1999).

Salt stress decreased chlorophyll as compared to the non-saline conditions. These results are similar to those of Downton *et al*. (1985), Stepien and Klobus (2006) and Yildirim *et al.,* (2008) who indicated that chlorophyll content significantly decreased in the leaves of spinach and cucumber plants with increasing NaCl concentration. In the present study, Si treated plants showed greater chlorophyll values than non-treated plants. These results are similar with Al-aghabary *et al*. (2004) that reported Si treatments caused to increase chlorophyll content of leaves of tomato under salt stress.

Salinity impairs membrane permeability and increases electrolyte leakage. However, application of Si partly maintained membrane permeability (Table 4B). Present study showed that Si reduced the amount of ion leakage in salt stressed calendula plants and Si protected the maintenance of membrane functions under stress conditions. Supporting evidence was shown when Si reduced electrolyte leakage salt stress condition in rice (Agarie *et al.*, 1998) leaves.

Salt stress decreased flower number per plant and flower diameter as compared to the non-saline conditions. In this experiment, different concentrations of Si increased flower number per plant and flower diameter. This is in agreement with the works by Savvas *et al*. (2002) and Kamenidou *et al*. (2010) that previously reported an increased gerbera flower quality when potassium silicate was added to the hydroponic nutrient solution. Hwang *et al.* (2005) also reported that applications of potassium silicate improved the growth and quality of cut flower miniature rose 'Pinocchio' in the rockwool culture system in agreement with the present results. It is possible that higher chlorophyll contents in Si treatments resulted in photosynthetic activity improvement and higher productivity (Table 4A).

CONCLUSION

Based on the present results, Si alleviates the negative effect of salt stress on growth, chlorophyll reading values, electrolyte leakage and flower quality depending on the concentration of Si Maximum alleviation of salt stress was found with 100mg/l Si application.

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