

ORIGINAL ARTICLE

Impact of exogenous salicylic acid on growth and ornamental characteristics of calendula (*Calendula officinalis* L.) under salinity stress

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Application of salicylic acid (SA) as a phytohormone has been increased due to resistance to stresses such as salt stress. Pot experiments were conducted to determine the effect of exogenous salicylic acid application on growth and ornamental characteristics of calendula grown under salt stress and greenhouse conditions. For this purpose a factorial experiment based on completely randomized design was conducted with 3 levels of SA (0 (control), 1, 2 mM) and 3 levels of NaCl (0, 100 and 200 mM) with 4 replications. At flowering stage, SA was applied with spraying two times in two week intervals. NaCl was also applied as drench (200 ml per pot) in two day intervals. The results showed that salinity decreased the growth, Chlorophyll reading values, flower number per plant and flower diameter. However, foliar applications of SA resulted in greater root, shoot and total dry weight, plant height and leaf area of calendula plants under salt stress. The highest chlorophyll reading values was obtained from 2.00 mM SA application in all NaCl treatments. Salinity decreased number of flower per plant and flower diameter as ornamental characteristics; however SA increased them under salinity stress. Plants treated with 1.00 mM SA had the highest flower diameter at 100 and 200 mM of NaCl. Electrolyte leakage increased by salinity, however foliar application of SA significantly reduced electrolyte leakage under salt stress. Based on the present results, foliar application of SA treatments can ameliorate the negative effects of salinity on the growth and ornamental characteristics of calendula plants.

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Salinity is one of the major environmental factors limiting plant growth and productivity. It is estimated that about one-third of world's cultivated land is affected by 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 (Alarc' on *et al.*, 1994) and even within a

given species (S' anchez-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 hyperionic and hyperosmotic 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 provides. It grows in sun or partial shade and is easy to grow requiring little cultivation (Dole and Wilkins, 2004).

Salicylic acid (SA), a naturally occurring plant hormone, acts as an important signaling molecule in plants, and has diverse effects on tolerance to abiotic stress (Bergmann *et al.*, 1994; Van Breusegem *et al.*, 2001). Exogenous application of SA may participate in the regulation of physiological processes in plants, such as ion uptake and transport, stomatal closure (Gunes *et al.*, 2005), membrane permeability (Barkosky and Einhellig, 1993) and photosynthesis and growth (Khan *et al.*, 2003). SA has been shown to play a role in mitigating the adverse deleterious effects of some environmental stresses including low temperature on banana (Kang, 2003), high temperature on (He *et al.*, 2005), drought stress on tomato (Hayat *et al.*, 2008) and wheat (Singh and Usha 2003), heavy metal on maize (Krantev *et al.*, 2008). The ameliorative role of SA to deleterious effects of salinity has been examined in different crops e.g. maize (Turan and Aydin 2005), tomato (Stevens *et al.*, 2006), barley (El-tayeb, 2005),

carrot (Eraslan, 2007) and wheat (Hamada and Al-Hakimi, 2001).

Water is becoming more and more rare resource 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 paper was to report the effects of SA on growth and quality flower of calendula grown under normal and salinity stress conditions.

MATERIALS AND METHODS

Plant material, growth conditions and treatments

Calendula seeds (*Calendula officinalis* L. cv. "Nana Bon Bon Naranja") were selected and germinated in coco peat in plastic germination trays under greenhouse conditions of Ferdowsi University of Mashhad (36° 17' 44" N and 59° 36' 42" E), Iran. Uniform size seedlings (40-day-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 SA (0 (control), 1, 2

mM) and 3 levels of NaCl (0, 100 and 200 mM) with 4 replications. Salicylic acid was dissolved in absolute ethanol then added drop wise to water (ethanol/water: 1/1000, v/v). Thirty days after transplanting, SA 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 day intervals. A control group of plants was grown without NaCl and sprayed with deionized water. All plants were harvested 60 d after planting (30 days after treatments) and separated into leaves, stem, and root.

Measurements and data collection

At the end of the experimental period, 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). 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 reading) 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). Plant height, flower diameter and number of flower per plant were also measured.

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.

Table 1: The dry weight of shoot, root and total plant of calendula in response to foliar SA applications under salt stress.

NaCl (mM)	SA (mM)	Shoot dry weight (g/plant)	Root dry weight (g/plant)	Total dry weight (g/plant)
0	0	5.7 cd	3.9 c	9.5 c
	1	7.0 b	5.0 a	12 b
	2	10.7 a	5.1 a	15.7 a
100	0	3.5 f	2.0 e	5.6 ef
	1	5.4 de	3.8 c	9.7 c
	2	6.8 bc	4.2 b	10 c
200	0	2.2 g	1.6 f	3.8 f
	1	4.3 ef	2.7 d	7.1 de
	2	5.9 bcd	2.8 d	8.6 cd
NaCl		**	**	**
SA		**	**	**
NaCl × SA		**	**	**

Numbers with the same letters in the same column are not statistically different ($P < 0.05$).

** : significant at 1% probability.

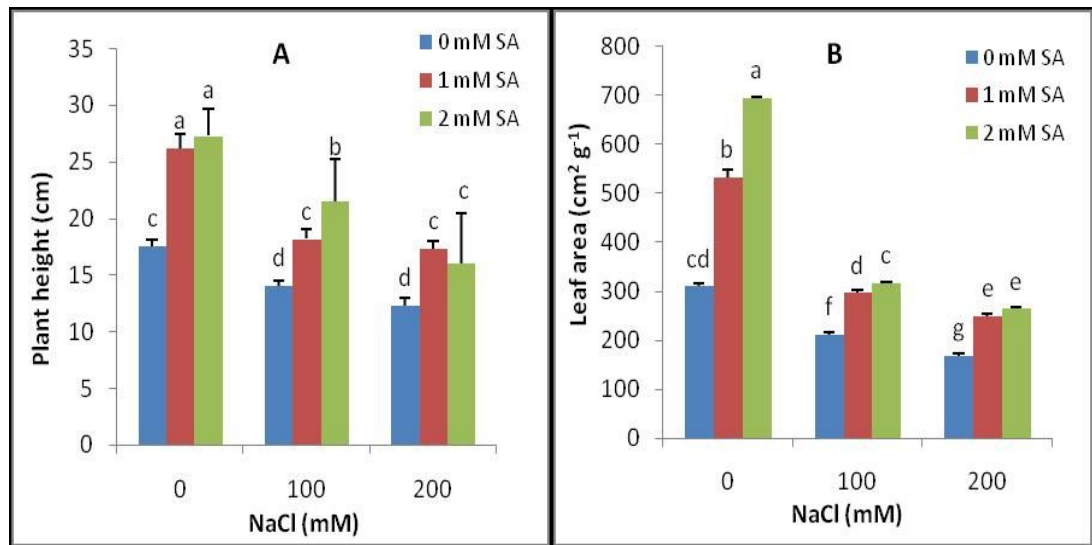


Figure 1. Plant height (A) and leaf area (B) of calendula plant in response to foliar SA applications under salt stress. Different letters on top of bars indicate significantly differences according to LSD test ($p < 0.05$) at each salt level. Vertical bars indicate the mean $\pm$ SE.

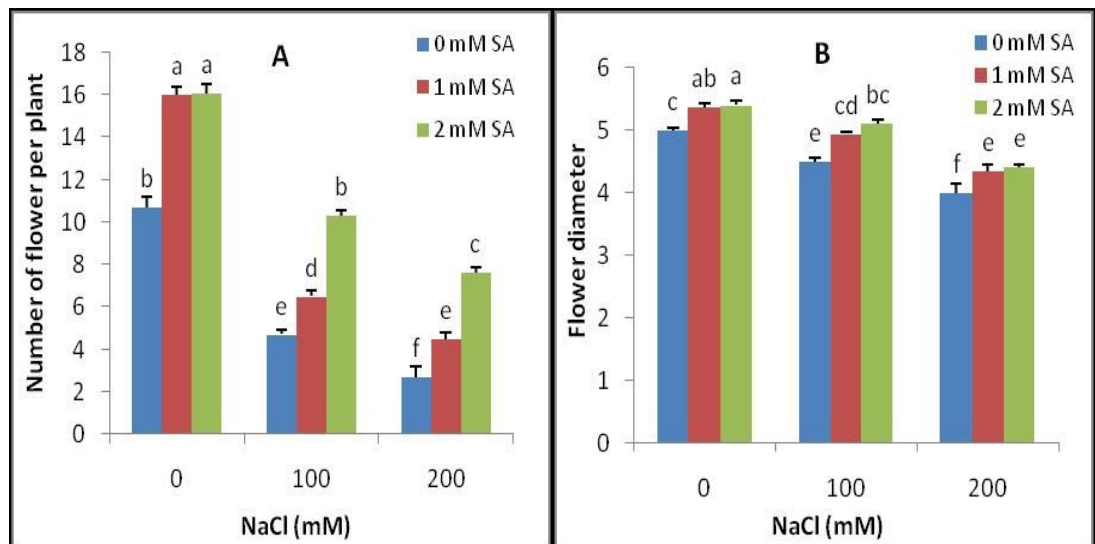


Figure 2. Flower number per plant (A) and flower diameter (A) of calendula plant in response to foliar SA applications under salt stress. Different letters on top of bars indicate significantly differences according to LSD test ($p < 0.05$) at each salt level. Vertical bars indicate the mean $\pm$ SE.

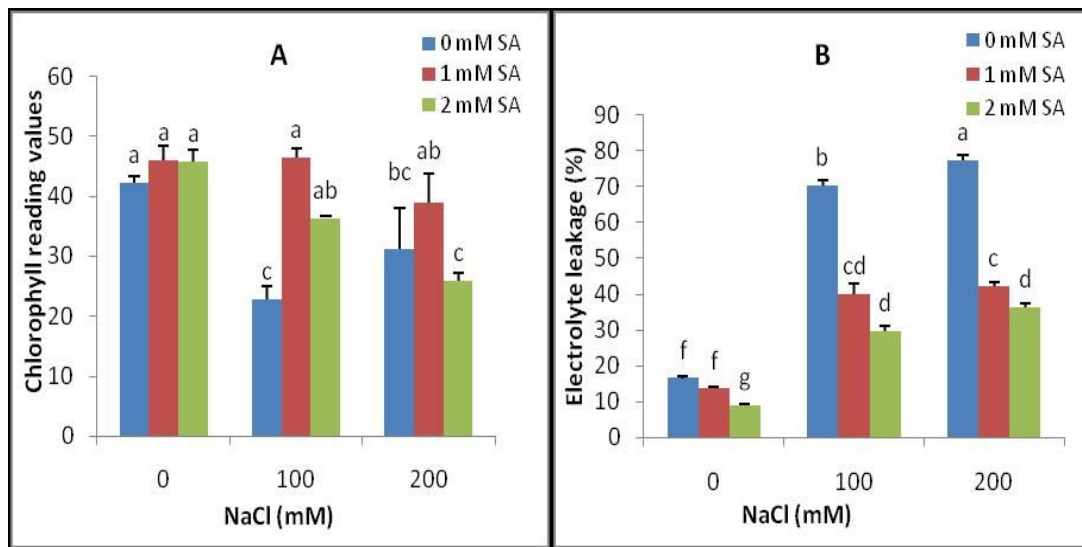


Figure 3. Chlorophyll reading values (A) and electrolyte leakage (B) of calendula plant in response to foliar SA applications under salt stress. Different letters on top of bars indicate significantly differences according to LSD test ($p < 0.05$) at each salt level. Vertical bars indicate the mean $\pm$ SE.

RESULTS

Plant growth

Shoot, root and total dry weight was recorded as influenced by different levels of SA and NaCl. As shown in table 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 SA increased dry matter accumulation in all parts of calendula plants under salt stress. Application of SA (2.00 mM) under salt stress (100 mM NaCl) gave the higher values for these parameters than the other treatments. It indicated that foliar application of SA alleviated the growth inhibition induced by added NaCl. Exogenous application of SA also enhanced shoot, root and total plant dry weight under no salt stress (Table 1).

Salt stress decreased leaf area as compared to the non-saline conditions. NaCl (200 mM) decreased leaf area by 45%, but the reduction was only 14% when 2 mM SA treatment was applied to NaCl-treated plants, as compared to control. Under non-saline conditions, foliar application of SA (2mM)

significantly increased leaf area (1.2 fold) as compared to control (Fig. 1A).

Salt stress significantly decreased plant height; however, foliar application of SA improved plant height under salt stress (Fig. 1B). Treatment 2 mM SA + 100 mM NaCl had the highest plant height as compared to the other treatment under salt stress. Foliar application of SA increased plant height by 55% as compared to control (Fig. 1B).

Flower number and flower diameter

Salt treatment (100 mM) significantly decreased number of flower per plant by 74% as compared to control, but the reduction was only 30% when SA (2mM) was applied to NaCl-treated plants. Under non-salt stress condition, SA increased flower number by 50% as compared to control (Fig. 2A).

Flower diameter of calendula plants decreased dramatically with the increasing NaCl concentration. All SA treatments increased the flower diameter compared to non-treated plants both in absence and presence of salinity. Plants treated with 1.00 mM SA had the highest flower diameter at 100 and 200 mM of NaCl (Fig. 2B).

Chlorophyll reading values

Chlorophyll reading values were significantly decreased with the increasing salinity stress. However, foliar SA applications used in the study caused to the elevated reading values. The highest reading values were obtained from 2.00 mM SA application in all NaCl treatments (Fig. 3A).

Electrolyte leakage

Based on the present results, electrolyte leakage was intensively increased by salt treatment; however, SA significantly decreased electrolyte leakage under salt stress. The lowest electrolyte leakage was obtained by 1.00 mM SA in 100 mM NaCl concentration under salt stress condition. Under no salt stress, foliar application of SA only slightly decreased electrolyte leakage compared to control (Fig. 3B).

DISCUSSION

Salinity decreased calendula plant growth significantly. Growth reduction under saline conditions has been well documented in various plants by many authors (Kaya *et al.*, 2003; Alpaslan and Gunes, 2001; Senaratna *et al.*, 2000; Turan and Aydin, 2005; Sivritepe *et al.*, 2003). As stated by Munns (2003), suppression of plant growth under saline conditions may either be due to decreased availability of water or to the toxicity of sodium chloride. SA treatments alleviated the deleterious effects of salinity on plant growth. Similar results were reported by Shakirova *et al.* (2003) in wheat, El-Tayeb (2005) for barley, Steven *et al.* (2006) and Szepesi *et al.* (2005) for tomato, Yildirim *et al.* (2008) for cucumber and Khodary (2004) for maize who observed exogenous SA treatments ameliorated the negative effects of salt stress plant growth. Gutiérrez-Coronado *et al.* (1998) reported that foliar application of SA significantly increased the growth of shoots and roots of soybean in either greenhouse

or field conditions. In ornamental plants, such as Gloxinia and violet, SA increased the number of leaves formed, and leaf area as compared to the control (Hayat and Ahmad, 2007; Martin-mex, R., 2005). This positive effect of SA could be related to an increased CO₂ assimilation and photosynthetic rate and increased mineral uptake by the stressed plant under SA treatment (Karlidage *et al.*, 2009; Khan *et al.*, 2003; Szepesi *et al.*, 2005). Moreover the induction of antioxidant response and protective role of membranes by SA caused increase the tolerance of plant to damage (Turan and Aydin, 2005).

Salt stress decreased chlorophyll reading values as compared to the non-saline conditions. These results are similar with 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, SA treated plants showed greater chlorophyll reading values than non-treated plants. These results support those of El-Tayeb (2005) and Yildirim *et al.* (2008) that reported SA treatments caused to the increased chlorophyll content of leaves of barley and cucumber under salt stress.

Salinity impaired membrane permeability increasing electrolyte leakage. However, application of salicylic acid (SA) partly maintained membrane permeability (Table 3B). Present study showed that SA reduced the amount of ion leakage in salt stressed calendula plants indicating that SA treatment has facilitated the maintenance of membrane functions under stress conditions. Supporting evidence was shown when SA reduced electrolyte leakage salt stressed tomato (Stevens *et al.*, 2006) and maize (Turan and Aydin, 2005) leaves.

Salt stress decreased flower number per plant and flower diameter as compared to the non-saline conditions. In this experiment plants treated with different concentration of SA, flowered early and their floral buds per plants were increased. SA has been shown to induce flowering in species of Lemnaceae like *oncidium*, an ornamental orchid species, *Impatiens balsamina*, *Arabiaopsis thaliana* and in *Pistia stratiotes* (Raskin, 1992) that are in agreement with the results of the present study. Moreover, salicylic acid also hastens flower initiation in *Saintpaulia ionantha* Wendl. (Martínez, 2005) and increased flower size in *Campanula* (Serek, 1992). Kumar & Nanda (1981a, 1981b) reported that the higher number of floral buds induced by salicylic acid was accompanied by increase in protein synthesis and appearance of new isozym bands.

Based on the present results, SA alleviates the negative effect of salt stress on growth, chlorophyll reading values, electrolyte leakage and flower quality depending on the concentration of SA used. Maximum alleviation of salt stress was found with 2 mM SA application.

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