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Research Article

RESPONSE OF SPECKLED SPUR-FLOWER TO SALINITY STRESS AND SALICYLIC ACID TREATMENT

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ABSTRACT

One of the limitations to using ornamental plants in green areas is too high salinity and alkalization of the soil. The adverse effect of salinity on plant growth and development may be effectively reduced by application of salicylic acid. *Plectranthus ciliatus* is an attractive bed plant with ornamental leaves, recommended for growing in containers, hanging baskets, or sunny borders. The aim of this study was to investigate the response of *P. ciliatus* to salicylic acid and calcium chloride. The plants were grown in pots in a glasshouse and were sprayed with solution of 0.5 mM salicylic acid and watered with 200 mM calcium chloride. The application of salicylic acid resulted in an increased weight of the aboveground parts, higher stomatal conductance and leaf greenness index and enhanced leaf content of nitrogen, potassium, iron and zinc. Salinity-exposed plants were characterized by reduced weight, stomatal conductance and leaf greenness index. Salt stress caused also a drop in leaf content of nitrogen, potassium and iron, and an increase in calcium, sodium, chlorine, copper and manganese concentration. Salicylic acid seemed to relieve salinity-mediated plant stress.

Keywords: *Plectranthus ciliatus*, ion concentration, purple-leaved plectranthus, salinity stress.

INTRODUCTION

There is an ongoing search for ornamental plant species that can be successfully grown in urban landscapes areas where soil salinity is high [Villarino and Mattson, 2011; Niu et al., 2012; Breś et al., 2014]. High salinity of the soil along transportation routes, resulting from using salt for deicing streets in the winter, is a considerable threat to plants [Jull, 2009]. Salinity inhibits plant growth and development, mainly by disturbing nutrient absorption, inducing osmotic stress and due to direct ion toxicity [Greenway and Munns, 1980]. Another adverse factor limiting the use of green areas is their alkalization, resulting from dust deposition and accumulation of construction debris that introduce large quantities of calcium compounds into the soil [Breś, 2008]. Plants exhibit different tolerance to salinity depending on their adaptation to specific habitat conditions [Niu and Rodriguez, 2006; Niu and Cabrera, 2010].

Salicylic acid (SA) is a phenolic compound and a natural growth regulator in vascular plants [Larque-Saavedra and Martin-Mex, 2007; Rivas-San Vicente and Plasencia, 2011]. It affects many physiological and metabolic processes [Jayakannan et al., 2015]. It also stimulates photosynthesis, transpiration, and ion uptake and transportation [Sahu, 2013]. Numerous studies have shown that exogenous salicylic acid induces plant resistance to abiotic stresses such as salinity, drought, heavy metals, too low or too high temperature or UV radiation [Yildirim et al., 2008; Li et al., 2014; Belkadhi et al., 2015; Khan et al., 2015; Taria et al., 2015]. Research literature does not provide any information regarding the use of salicylic acid in the cultivation of ornamental bedding plants, especially those exposed to salt stress.

Plectranthus L'Hér. (Lamiaceae) is a horticulturally important genus of about 300 species grown for foliage and flowers [Rice et al., 2011]. Some species are useful outdoors in large containers, hanging baskets, or sunny borders. *Plectranthus ciliatus* E.Mey. ex Benth. is a native South Africa species [Potgieter et al., 1999]. It is a valuable ornamental plant with delicate white flowers and attractive leaves with green upper surface and purple lower surface [Bercu, 2013]. *P. ciliatus* can be grown in gardens and green areas. The species has also medicinal properties [Stavri and Gibbons 2007]. The effect of exogenous salicylic acid and salinity on the growth of *P. ciliatus* has not been examined so far.

The aim of this study was to compare changes in leaf greenness index and stomatal conductance, and leaf content of macro- and micronutrients in *P. ciliatus* exposed to salinity stress and treated with salicylic acid.

MATERIALS AND METHODS

The plant material included 10 cm rooted cuttings of *Plectranthus ciliatus* E.Mey. ex Benth. The plants were planted on 16 April 2014 into PCV pots with a capacity of 2.5 dm^3. Peat substrate (pH 6.0) was mixed with Azofoska fertilizer (13.6% N, 6.4% P_2O_5, 19.1% K_2O, 4.5% MgO, 23.0% SO_3, 0.045% B, 0.18% Cu, 0.17% Fe, 0.27% Mn, 0.04% Mo, and 0.045% Zn) at a dose of 2.5 g·dm^{-3} of the substrate. The pots were placed on tables in a greenhouse belonging to the West Pomeranian University of Technology in Szczecin (53° 25' N, 14° 32' E).

The experimental variants were as follows:

- control – no treatment,
- spraying with salicylic acid solution,
- watering with $CaCl_2$ solution,
- spraying with salicylic acid solution and watering with $CaCl_2$ solution.

Each experimental variant included 16 plants, four plants per repetition. Salicylic acid (Sigma Aldrich, USA) was dissolved in small amount of 99.8% ethanol (Chempur, Poland). The plants were sprayed with 0.5 mM SA four times, every five days starting from 10 May 2014. One plant was sprayed with about 10 cm^3 of the solution. The solution was supplemented with Silwet Gold wetting agent (Momentive Performance Materiale, USA). The plants exposed to salinity stress were watered with 200 mM $CaCl_2 \times 2H_2O$ (Chempur, Poland) four times, every five days from 1 June 2014. Each time one plant in a pot was watered with 200 ml of the salt solution. The plants were cultivated until 10 September 2014 under natural photoperiod. They were watered three times a week with tap water with EC 6.5. Temperature and relative humidity were recorded over the entire cultivation period by datalogger Testo-175-h2 (Table 1).

Physiological parameters were assessed in the first decade of September. Leaf greenness index (SPAD) was measured with an optical device Chlorophyll Meter SPAD 502 (Minolta, Japan). The measurements included five leaves located in the middle of a plant and three readings were taken per each leaf. Stomatal conductance was assessed with SC1 porometer (Dekagon Devices, USA). The measurements were performed only on sunny days between 10 a.m. and 12 p.m., and included five leaves per plant. Plant height, width and fresh weight of aboveground part were estimated on the last day of the cultivation. The collected leaves were dried, ground and sent to an accredited laboratory of the Chemical and Agricultural Station in Szczecin for determination of macro- and micronutrient content according to standard methods [Ostrowska et al., 1991]. Leaf chemical analyses were repeated three times for each experimental variant.

Statistical analysis of the results involved a univariate analysis of variance. Mean values were compared using Tukey test for a significance level $P = 0.05$.

Table 1. The air temperature (ºC) and relative humidity (RH %) during the experiment

Parameter		Month					
		April	May	June	July	August	September
ºC	Min.	7.57	6.9	13.3	17.6	14.7	13.0
	Average	15.1	18.9	19.7	24.1	19.6	18.3
	Max.	23.8	25.0	27.3	32.5	25.6	25.9
RH %	Min.	31.0	46.3	45.3	41.7	45.9	37.0
	Average	62.0	76.9	70.6	69.4	78.5	80.4
	Max.	86.9	98.5	98.5	99.3	97.7	99.7

RESULTS AND DISCUSSION

The effects of SA and $CaCl_2$ on the growth, leaf greenness index and stomatal conductance of *P. ciliatus* are presented in Figures 1–5. Apparently, SA and $CaCl_2$ had no effect on *P. ciliatus* diameter (Figure 1) but both treatments affected plant height (Figure 2). The control plants were the highest, and the experimental ones were significantly shorter and of similar height (Figure 2). Growth inhibition of salt-treated plants has been widely reported by many authors [Niu et al., 2010,

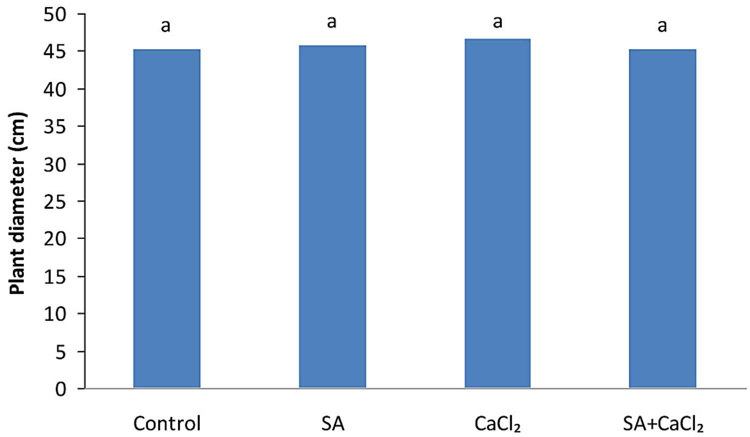

Figure 1. Effect of calcium chloride ($CaCl_2$) and salicylic acid (SA) on plant diameter of *Plectranthus ciliatus*. Means followed by the same letters are not significantly different, tested by Tukey's multiple comparison at $P = 0.05$

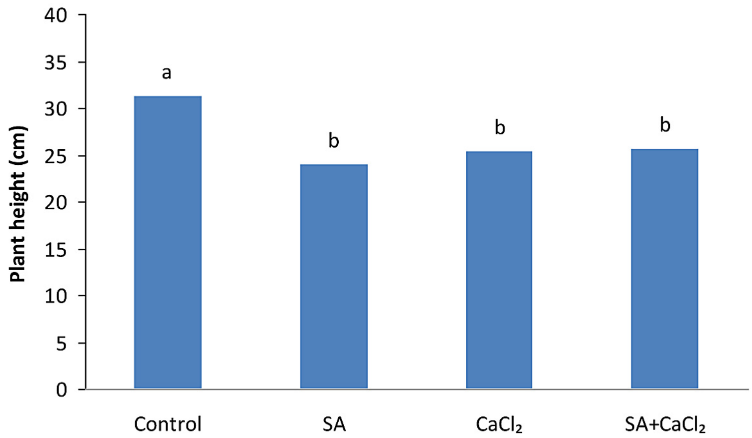

Figure 2. Effect of calcium chloride ($CaCl_2$) and salicylic acid (SA) on plant height of *Plectranthus ciliatus*. Means followed by the same letters are not significantly different, tested by Tukey's multiple comparison at $P = 0.05$

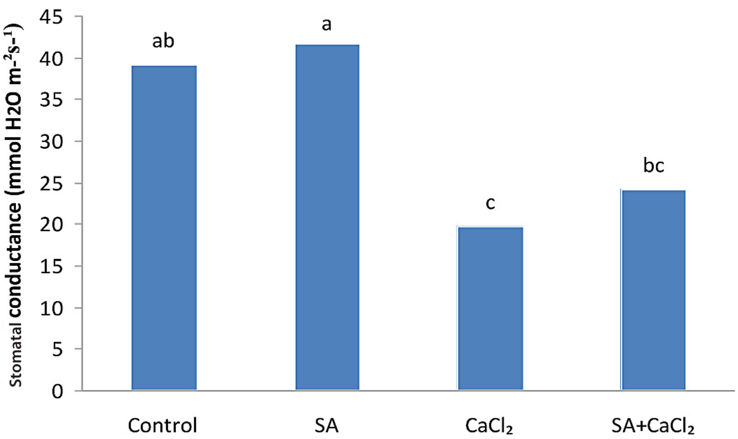

Figure 3. Effect of calcium chloride ($CaCl_2$) and salicylic acid (SA) on stomatal conductance of *Plectranthus ciliatus*. Means followed by the same letters are not significantly different, tested by Tukey's multiple comparison at $P = 0.05$

Gao et al., 2012, Cai et al., 2014]. Plants treated with salicylic acid are usually higher [Gunes et al., 2007], but in some species SA treatment may restrict plant growth, especially when used at higher concentrations [Kovácik et al., 2009; Jayakannan et al., 2015].

The study investigated changes in stomatal conductance to figure out plant response to the growth stimulator and salt stress (Figure 3). The highest stomatal conductance was measured in the plants sprayed with SA (41.7 mmol H_2O m^{-2} s^{-1}), thus indicating a stimulating effect of this compound on the intensity of photosynthesis. In the plants treated with SA + $CaCl_2$, stomatal conductance was by 4.5 mmol H_2O m^{-2} s^{-1} higher than in those treated with $CaCl_2$ alone. The lowest stomatal conductance (19.7 mmol H_2O m^{-2} s^{-1}) was read in the plants treated with $CaCl_2$ alone (Figure 3). Our results confirm the reports of other authors on adverse effects of salinity on water management in plant cells and tissues [Bãnón et al., 2011]. Stomata are primary elements controlling leaf surface conductance and they regulate both water loss and CO_2 assimilation during photosynthesis. Therefore, stomatal conductance is an important parameter of plant water status and it provides important information on plant growth and adaptation to variable environmental conditions [Arve et al., 2011].

The plants treated with salicylic acid were found to have the highest greenness index (Figure 4). A positive effect of SA on leaf chlorophyll content has also been shown by Li et al. [2014] in soybean seedlings. The lowest greenness index was noticed in the plants exposed to salinity stress (Figure 4). This finding was consistent with the data reported by Freitas et al. [2014], who described a drop in the greenness index in

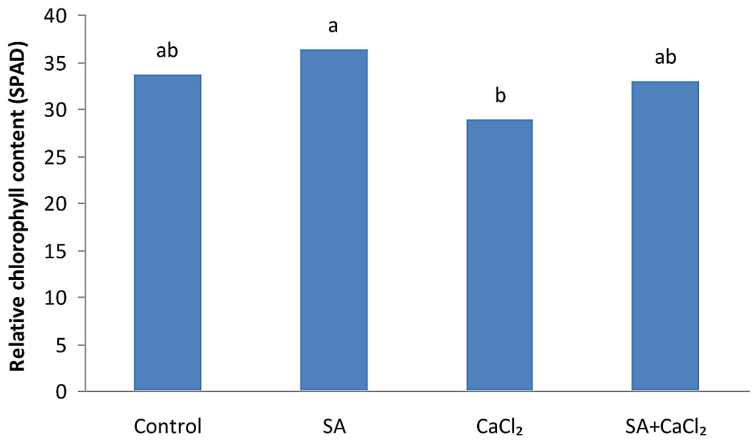

Figure 4. Effect of calcium chloride ($CaCl_2$) and salicylic acid (SA) on relative chlorophyll content of *Plectranthus ciliatus*. Means followed by the same letters are not significantly different, tested by Tukey's multiple comparison at $P = 0.05$

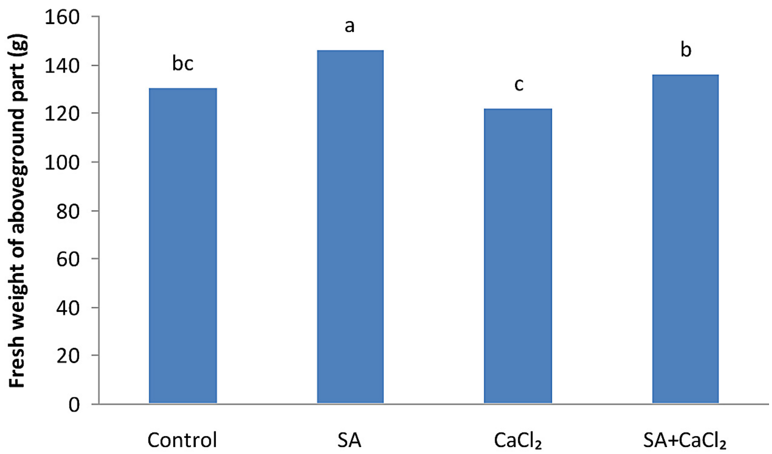

Figure 5. Effect of calcium chloride ($CaCl_2$) and salicylic acid (SA) on fresh weight of aboveground part of *Plectranthus ciliatus*. Means followed by the same letters are not significantly different, tested by Tukey's multiple comparison at $P = 0.05$

Plectranthus amboinicus, *Plectranthus barbatus* and *Plectranthus grandis* watered with water with EC 0.7–5.5 dS m^{-1}. They also reported *P. amboinicus* as a species most sensitive to salinity. Spraying $CaCl_2$-treated plants with SA increased the greenness index that was by four SPAD units greater than in the plants treated with $CaCl_2$ alone (Figure 4). Salt stress is known to inhibit nitrogen assimilation and disrupt protein metabolism [Starck 2005]. Chlorophyll synthesis is disturbed, which causes yellowing of leaves and tissue necrosis manifested by necrotic spots [Niu and Cabrera, 2010].

The plants treated with salicylic acid had the highest fresh weight of the aboveground part (Figure 5). Increased biomass resulting from SA treatment was also reported in *Plectranthus tenuiflorus* [Jalal et al., 2012]. The lowest fresh weight of the aboveground part was found in *P. ciliatus* watered with $CaCl_2$ (Figure 5). A significant decrease in the weight of plants exposed to salinity stress was also observed in other three *Plectranthus* species, i.e. *P. amboinicus*, *P. barbatus,* and *P. grandis* [Freitas et al., 2014]. Fresh weight of the plants treated with SA + $CaCl_2$ was by 14 g greater than of those treated with $CaCl_2$ alone (Figure 5). This seems to confirm previous reports on protective role of SA in plants exposed to salt stress [Manaa et al., 2014].

Date in Table 2 show the content of macronutrients in the leaves of *P. ciliatus* treated with salicylic acid and sodium chloride. Laboratory analyses revealed the highest content of nitrogen in the leaves sprayed with SA. Nitrogen is a macronutrient strongly affecting plant growth and yield and this is probably why plants treated with SA produced the highest biomass. Salicylic acid spraying resulted also in a significant increase in leaf potassium concentration. These findings were consistent with those of Amir et al. [2014] and Mirdad [2014], who reported enhanced content of nitrogen and potassium in SA-treated grapevine and broccoli.

The lowest content of N and K was found in the plants watered with $CaCl_2$ (Table 2). These results confirmed the findings of Freitas et al. [2014], who described reduced potassium concentration in the leaves of *P. amboinicus*, *P. barbatus* and *P. grandis* exposed to salt stress. Literature reports indicate that excessive concentration of ions, especially Na^+ and Cl^- may limit the uptake of other ions, particularly K^+ and Ca^{2+} [Marschner, 1995]. Potassium is an essential ion determining cellular osmotic potential and stomata functioning [Oosterhuis et al., 2014]. In this study, the salt-treated plants had low stomatal conductance that might be due to reduced potassium supply.

Sodium leaf content was higher in salt and salt + SA treated plants, as compared with control plants and those sprayed with SA alone (Table 2). The plants watered with $CaCl_2$ and those treated with $CaCl_2$ + SA contained over six times more chlorine than the controls and the plants sprayed with SA alone. The highest calcium concentration was found in the plants watered with $CaCl_2$, and the lowest in the control plants. Increased content of calcium and chlorine in the plants exposed to salt stress probably resulted from using $CaCl_2$ solution.

No effects of salt or SA on the leaf concentration of phosphorus or magnesium were detected (Table 2), and their content ranged from 0.20 to 0.32% DW (P) and from 0.70 to 0.77% DW (Mg).

The data recorded in Table 3 present the effects of SA and $CaCl_2$ on micronutrient concentration in the leaves of *P. ciliatus*. Its leaves were characterized by relatively high concentration of iron, as compared with other micronutrients, which is a common situation in plants. The highest iron content was found in SA-treated plants. Increased Fe concentration resulting from SA application was also reported in *Arachis hypogaea* [Kong et al., 2014]. The lowest Fe level was detected in the leaves of plants watered with $CaCl_2$. These results confirmed the findings of Bhatt et al. [2008], who reported a significant decrease in Fe in the leaves of salt-treated *Ziziphus mauritiana.* Iron is located mainly in chloroplasts, where

Table 2. Effect of salicylic acid (SA) and calcium chloride ($CaCl_2$) on the concentrations (% dry weight) of macronutrients in the leaves of *Plectranthus ciliatus*. Means (n = 3) within each column followed by the same letter are not significantly different, tested by Tukey's multiple comparison at P = 0.05

Treatments	N	P	K	Ca	Mg	Na	Cl
Control	0.61 ^{ab}	0.27 ^{a}	1.00 ^{ab}	1.57 ^{c}	0.77 ^{a}	0.24 ^{b}	0.64 ^{b}
SA	0.85 ^{a}	0.20 ^{a}	1.41 ^{a}	2.69 ^{b}	0.70 ^{a}	0.27 ^{b}	0.59 ^{b}
$CaCl_2$	0.57 ^{b}	0.31 ^{a}	0.86 ^{b}	4.65 ^{a}	0.75 ^{a}	0.31 ^{a}	4.19 ^{a}
$CaCl_2$ + SA	0.65 ^{ab}	0.32 ^{a}	0.99 ^{ab}	3.97 ^{ab}	0.74 ^{a}	0.31 ^{a}	4.05 ^{a}

Table 3. Effect of salicylic acid (SA) and calcium chloride ($CaCl_2$) on the concentrations micronutrients (mg/kg dry weight) in the leaves of *Plectranthus ciliatus*. Means (n = 3) within each column followed by the same letter are not significantly different, tested by Tukey's multiple comparison at *P* = 0.05

Treatments	Fe	Mn	Cu	B	Zn
Control	902 ^{b}	253 ^{b}	5.51 ^{b}	34.6 ^{b}	104 ^{b}
SA	1125 ^{a}	223 ^{b}	5.31 ^{b}	24.0 ^{c}	119 ^{a}
$CaCl_2$	621 ^{d}	357 ^{a}	7.14 ^{a}	26.4 ^{c}	110 ^{ab}
$CaCl_2$+SA	866 ^{c}	334 ^{a}	8.21 ^{a}	45.1 ^{a}	117 ^{a}

it acts as a stimulator of synthesis of chlorophyll and some other proteins [Briat et al., 2015]. Low greenness index observed in *P. ciliatus* plants may be correlated with low leaf iron content.

Manganese concentration was significantly higher in the plants treated with $CaCl_2$ and SA + $CaCl_2$ (Table 3). Increased Mn leaf content was also reported in salt-treated *Ziziphus mauritiana* [Bhatt et al., 2008]. Manganese activates many enzymes and it is involved in the release of O_2 during photosynthesis [Marschner, 1995]. Elevated concentration of Mn in *P. ciliatus* plants watered with $CaCl_2$ might indicate their tolerance to salinity stress.

Plant tissues require maintaining a specific ratio of iron and manganese (Fe/Mn). In our study, mean Fe/Mn ratio was 2.2 and it was lower than that reported for *Lamiaceae* family (Fe/Mn above 4) encompassing *P. ciliatus.* However, Fe/Mn ratio may vary from below 1.0 to over 4.0 and it depends on taxonomic features [Starck, 2005].

Leaves of the plants treated with $CaCl_2$ and SA + $CaCl_2$ contained significantly more copper as compared with the control plants and those sprayed with SA alone (Table 3). Copper plays an important role in photosynthesis and respiration, in the metabolism of sugars and nitrogen compounds, and in cell wall lignifications [Starck, 2005]. Increased copper content in plants grown under salt stress may be due to a participation of this micronutrient in the process of adaptation to these conditions [Waraich et al., 2011].

The highest boron content was found in salt + SA treated plants (Table 3). The lowest boron content was measured in plants treated with $CaCl_2$ or SA. Boron is involved in the formation of cell wall structures, plant growth, and indirectly in carbohydrate metabolism [Starck, 2005].

Higher concentration of zinc was detected in the plants sprayed with SA and sprayed with SA and watered with $CaCl_2$ (Table 3). The lowest zinc content was measured in the control plants. An increase in Zn concentration following SA application was also reported in peppers [El-Yazied, 2011] and *Arachis hypogaea* [Kong et al., 2014]. Zinc plays different roles in plant vital processes, it activates the enzymes involved in sugar and protein metabolism, stabilizes protein structure and controls gene expression [Sadeghzadeh and Rengel, 2011].

CONCLUSIONS

1. Salicylic acid treatment increased plants' fresh weight, stomatal conductance, leaf greenness index and leaf content of nitrogen, potassium and iron.
2. Salinity stress reduced plant weight, stomatal conductance and leaf greenness index. Moreover, the leaves of salt-exposed plants contained less nitrogen, potassium, and iron and more calcium, sodium, chlorine, copper, and manganese.
3. Salicylic acid relieved the adverse effects of $CaCl_2$, as demonstrated by increased plant fresh weight, stomatal conductance and leaf greenness index compared to plants treated with $CaCl_2$ alone.

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