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Research Paper

Morphological and Physiological Traits of *Catharanthus roseus* L. at Different Irrigation Intervals as Affected by Salicylic Acid Application

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Abstract

The effects of varying irrigation intervals (I) and foliar application of salicylic acid (M) on morphological and physiological traits of *Catharanthus roseus* L. were studied in a factorial experiment based on a randomized complete design with three replications. The experimental treatments consisted of four irrigation intervals [2 days (I_1), 4 days (I_2), 6 days (I_3) and 8 days (I_4)] and foliar application of 200 mg L^{-1} salicylic acid (SA) at three frequencies [0 (M_0), one (M_1) and two (M_2) times]. Among the morphological traits, treatment $I2M_2$ resulted in the greatest number of flowers (25.66 flowers), the fewest leaf abscissions (3.8), and the highest root fresh weight (1.181 g); I_1M_0 resulted in the highest plant height (154 cm), leaf number (36 leaves), internode length (9.243 mm), shoot fresh weight (8.636 g), and shoot dry matter (26.20%); and I_1M_2 resulted in the largest flower diameter (1.176 mm), node number per plant (16.66 nodes), plant fresh weight (9.366 g), and root dry matter (30.03%). Among physiological traits, I_1M_0 was related to the highest chlorophyll b, total chlorophyll, and petal anthocyanin. The highest and lowest proline contents were obtained from I_3M_2 and I_2M_0, respectively. The lowest MDA content of 1.42 nmol g^{-1} fresh weight (FW) was observed in I_4M_1 and the highest SOD activity of 88 IU g^{-1} FW was obtained from I3M0. POD activity was lowest in I_2M_0 and highest in I_4M_2. In total, given the detrimental impacts of water deficit stress at irrigation intervals of 6 and 8 days, it is recommended that SA be applied to improve the growth of *Catharanthus roseus* L.

Keywords: Environmental stresses, Growth regulators, Ornamental-medicinal herb, Water deficit.

INTRODUCTION

Catharanthus roseus L., commonly known as the Madagascar periwinkle, is a plant from the family Apocynaceae. It is a precious medicinal herb because of its alkaloid and anti-cancer compounds including vincamine, isovincamine, and vincine (Sain and Sharma, 2013; Loyola-Vargas *et al*., 2007; van der Heijden *et al.*, 2004). It is also a popular ornamental plant (Jalili Marandi, 2010).

Water deficit stress has destructive effects on the physiological and morphological processes of the plants and is recognized as the most important environmental factor limiting plant growth and development. Plants can resist water deficit stress to a certain extent, but prolonged exposure can severely impair their growth and yield. Given the trend of increasing drought in the world, it is very important to provide solutions to increase the tolerance and resistance of plants to water deficit stress. In addition to the genetic breeding of plants to cope with environmental stresses, the use of chemicals, including growth regulators, is recommended since they are more economical and easier to use (Osakabe *et al*., 2014; Nazar *et al*., 2015; Elgamaal and Maswoda, 2013).

Salicylic acid (SA; $C_2H_6O_3$) is a phenol-based plant growth regulator that has antioxidant properties. This plant hormone plays a remarkable role in germination, vegetative and reproductive growth, photosynthesis, transpiration, nutrient uptake and mobilization, senescence, and induction of resistance to environmental stresses in different plants (Horváth *et al*., 2007; Bezrukova *et al*., 2001; Kareem *et al*., 2017). The role of SA has been proven in inducing drought resistance (Munne-Bosch and Penuelas, 2003; Chini *et al*., 2004).

Kareem *et al*. (2017) found that the water deficit significantly reduced the growth rate and yield of wheat, but that the foliar application of SA and molybdenum (Mo) increased the growth and yield in plants affected by drought stress. They reported that SA was more effective than Mo in alleviating the harmful impacts of drought stress and that SA application at low rates resulted in more positive effects on physiological properties, yield, and growth parameters of wheat. Elgamaal and Maswoda (2013) suggested that SA applied at the rates of 0.5 and 1 mM alleviated the undesirable effects of drought and improved the physiological characteristics and yields of yellow maize (*Zea mays* L.) under severe water deficit stress.

The effects of the growth regulators SA and ascorbic acid were studied on two sunflower hybrids under water deficit stress (Ahmed *et al*., 2014). These authors found that the water deficit significantly reduced germination, stem length, and fresh and dry weight; however, the application of 100 or 200 mg L^{-1} SA and ascorbic acid significantly reduced the harmful effects of drought and enhanced these traits. Salicylic acid was found to be more effective than ascorbic acid in improving the traits (Ahmed *et al*., 2014). There are further reports of the impact of SA on the improvement of the physiological and morphological traits of *Tagetes erecta* (Sandoval Yepiz, 2004), wheat (Hussein *et al*., 2007), rice (Farooq *et al*., 2010) and strawberries (Ghaderi *et al*., 2015).

A study on the effect of three SA application rates (0, 1 and 2 mM) on morphological and ornamental traits of *Petunia hybrida* under drought stress (FC of 40 and 70%) showed that higher levels of drought stress resulted in the loss of flower number, flower diameter, leaf area, root and branch fresh and dry weight, chlorophyll content, and stomatal conductance and an increase in electrolyte leakage, but the application of SA reduced the destructive effects of water deficiency and higher SA rates enhanced the morphological and ornamental traits of *P. hybrida* while reducing electrolyte leakage (Zarghami *et al*., 2014). Hosseini *et al*. (2015) reported that foliar application of SA to *Lolium* grasses affected by water deficiency improved chlorophyll a and b content and reduced electrolyte leakage. These authors also observed that the application of SA reduced the accumulation of proline amino acids and the activity of antioxidant enzymes, thereby minimizing the damage of drought stress to the plant.

Since *Catharanthus roseus* is a tropical plant and is highly likely to experience prolonged

water deficit, we explored the effect of foliar application of SA on its morphological and physiological traits when grown under a range of irrigation intervals.

MATERIALS AND METHODS

In February 2017, F1 seeds of *Catharanthus roseus* were sown in a 70% cocopeat + 30% perlite substrate and were kept in a plastic greenhouse at 15 °C and 80–90% relative humidity. The seeds were procured from Goldsmit Seeds Co. One month later, the two- to four-leaf seedlings were transferred to the main pots, which had a mouth diameter of 8 cm. After the warming of air for a further 30 days, they were moved out of the greenhouse into the open air where they were kept at an average temperature of 30°C for 90 days.

The study was carried out as a factorial experiment based on a randomized complete design with three replications. The twelve treatments including irrigation intervals at four levels [two days (I_1), four days (I_2), six days (I_3) and eight days (I_4)] and foliar application of 200 mg SA at three frequencies [Zero (M_0), one (M_1) and two (M_2) times]. Irrigation intervals were specified using a digital tensiometer (Germany). Irrigation practice as per the treatments commenced one week after the establishment of the plants in the main pots. The first foliar application of SA took place 15 days after transplanting the seedlings, and the second application took place 25 days after transplantation. After the seedlings were established in the main pots, they were fertilized with KRISTALON™ (20–20–20) once 20 days.

The assessment of morphological traits

The number of flowers per plant was averaged for three plants per plot from the beginning of flowering until 50% of the flowers had wilted. Plant height, number of nodes, internode length, and root length were measured on three plants per plot at the end of the experiment. Flower diameter was measured with a digital caliper on fully open flowers of three plants per plot during the experiment. At the end of the experiment, plant, shoot and root fresh weights (FW) were measured using a 0.01-precision digital scale on three plants per plot. Plants were then oven-dried at 70 °C for 48 hours to calculate the dry matter percentage of each organ through dividing dry weight by fresh weight and multiplying by 100 (Dashtbany *et al*., 2015).

The assessment of physiological traits

Before the end of the experiment, samples were taken to measure physiological traits.

Chlorophyll

The procedure of Mazumdar and Majumdar (2003) was used to measure chlorophyll a, b and total chlorophyll contents. To this end, the fresh leaves were extracted using 80% acetone and it was read at 660 and 643 nm with a spectrophotometer (Apel-PD-303 UV). The following equations were employed to determine chlorophyll a, b and total chlorophyll in mg g^{-1} FW:

$$\text{Chlorophyll a} = 9.93(A_{660}) - 0.777(A_{643})$$
$$\text{Chlorophyll b} = 17.6(A_{643}) - 2.81(A_{660})$$
$$\text{Total chlorophyll} = 7.12(A_{660}) + 16.8(A_{643})$$

Petal anthocyanin

To measure petal anthocyanin content, 0.5 g of fresh petal tissue was extracted with acidic methanol (pure methanol + hydrochloric acid) to be used for the measurement of anthocyanin content at 535 nm with a spectrophotometer. Anthocyanin content was obtained from the following formula (Mazumdar and Majumdar, 2003):

$$\text{Anthocyanin (mg/100 g FW)} = \frac{e \times b \times c}{d \times a} \times 100$$

where e = sample weight, b = sample volume for measurement, c = total volume of solution, d = sample volume, and a = the read figure.

Proline

Proline content was measured using the method of Bates *et al.* (1973). An extract of 0.5 g of dry matter was prepared with 10 ml of 3% sulfosalicylic acid and centrifuged at 6500 rpm for 20 minutes. Two milliliters of centrifuged extract were added to 10 ml distilled water, 2 ml ninhydrin acid and 2 ml glacial acetic acid, and the samples placed in a 100 °C hot bath for 90 minutes. After cooling, 4 ml toluene was added to each sample and the samples were shaken for 30 minutes. The clear surface layer of the samples was read at 520 nm with a spectrophotometer and the amount of proline was obtained in mg g^{-1} FW with the aid of a standard curve.

Malondialdehyde (MDA)

Malondialdehyde was used to measure lipid peroxidation, as described by Heath and Parker (1968). A sample of 0.5 g of fresh petal tissue was extracted using liquid nitrogen and 1 ml 50 mM potassium phosphate buffer (pH = 7) containing 0.5 M EDTA. This was centrifuged at 4 °C and 14000 rpm for 20 minutes. The resulting surface solution was separated with a sampler and re-centrifuged at 10500 rpm at the same temperature for 10 minutes. A 200 µl sample of surface solution was then added to 1000 µl 20% trichloroactic acid (TCA) containing 0.5% TBA. The resulting mixture was placed in a boiling water bath at 95 °C for 30 minutes and then immediately frozen in ice. The samples were subsequently centrifuged again at 10 500 rpm for 10 minutes. The absorption of reddish malondialdehyde-thiobarbituric acid (MDA-TBA) was read at 532 nm with a spectrophotometer (PG Instruments, T80), and the absorption of other specific pigments was read at 600 nm, and then it was subtracted from this value. MDA concentration was expressed in nmol g^{-1} FW.

Peroxidase (POD)

The extracts that were prepared in the MDA section were used in this assay. To evaluate the activity of the peroxidase enzyme, 450 µl H_2O_2 solutions (225 mM) and 450 µL guaiacol solution (225 mM) were mixed together at a low temperature (ice-containing container) and 100 µl enzyme extract was added. The absorption variations were then traced at 470 nm with a spectrophotometer (PG Instruments, T80). In the control solution, 100 µl of 50 mM phosphate buffer (pH = 7) was used instead of the enzyme extract. Enzyme activity was expressed in nmol g^{-1} FW (In *et al*., 2007).

Superoxide dismutase (SOD)

Superoxide dismutase activity was measured by spectrophotometry using the method of Giannopolitis and Ries (1997). Initially, 0.5 g frozen petal tissue was mixed with 1 ml 0.5 M potassium phosphate buffer and 0.1 g polyvinylpolypyrrolidone (PVPP) at pH = 7. After homogenizing (IKA-T8 Homogenizer, Germany), the samples were centrifuged at 4 °C and 14000 rpm for 15 minutes. The surface solution was removed slowly and immediately transferred to –80 °C. The reaction solution consisted of 0.1 mg EDTA, 50 mM phosphate buffer, 13 mM methionine, 75 mM NBT, and 2 mM riboflavin (1 ml total), and 100 µl enzyme extract. The tubes containing the reaction solution were exposed to fluorescence light (a 400-Lux fluorescent lamp) at 22 °C for 15 minutes while being shaken slowly, after which the absorbance of the samples was read at 560 nm. The activity of the enzyme was determined in IU g^{-1} FW.

Data analysis

Data were analyzed using the MSTATC Statistical Software Package and means were compared using the (least significant difference) LSD method.

RESULTS

Morphological traits

Analysis of variance showed that the interaction between irrigation interval and SA application (I × M) changed flower number, plant height, leaf number per plant, number of nodes, leaf abscission, plant, shoot and root dry matter, root fresh weight and root length significantly at the one percent level. The interaction I × M was also significant for flower diameter, internode length, plant fresh weight, and shoot fresh weight at the 5 percent level (Table 1).

Since the interaction I × M was significant for all morphological traits, the effects of individual treatments are only presented in tables 2 and 3.

Comparison of the means for all treatments (Table 4) shows that the number of flowers per plant was greatest (25.66 flowers) in treatment I_2M_2 and least (8.83 flowers) in I_4M_0. The greatest flower diameter of 1.176 mm was observed in plants treated with I_1M_2, though this value did not differ significantly from those of I_2M_2 (1.124 mm), I_1M_1 (1.084 mm) or I_1M_0 (1.054 mm). The smallest flowers (0.636 mm in diameter) were observed on plants in treatment I_4M_0. With an irrigation interval of 8 days, all three levels of SA application resulted in plant heights that were the smallest measured among all treatments. The largest plant height of 154 mm was observed in I_1M_0 (Table 4).

As is evident in Table 4, the smallest number of leaves grew on plants in I_4M_1 (16.33 leaves) followed by plants in I_4M_2 (18.66 leaves). Treatments I_1M_0 and I_2M_2 did not exhibit statistically significant differences and they were associated with the largest number of leaves (36 and 35 leaves respectively; Table 4).

At all four irrigation intervals, one-time application of SA reduced the number of nodes per plant versus control plants, but the number of nodes was increased with a two-time application of SA. With respect to internode length, the longest was 9.243 mm observed in I_1M_0, but this did not differ significantly differ from other treatments except for I_4M_2 and I_4M_1 which had the shortest internode lengths of 6.687 and 7.033 mm respectively (Table 4).

Leaf abscission was alleviated by SA application at all four irrigation intervals. The lowest leaf abscission (3.8) was observed in I_2M_2. Treatments I_3M_0 and I_2M_0 had the highest leaf abscission of 7.4 and 6.966 respectively. Plant fresh weight decreased with a decrease in irrigation frequency; the lowest plant fresh weight of 5.724 g was recorded in I_4M_1, but this was not significantly different from the fresh weight recorded inI_4M_2 (6.066 g). The highest plant fresh weight was observed for plants in treatment I_1M_2 (9.366 g) and I_1M_0 (9.214 g) The difference between these two treatments was not statistically significant (Table 4).

Comparison of means for plant dry matter showed that at irrigation intervals of 2, 4 and 6 days, a one-time application of SA was related to a greater dry matter than a two-time application of SA. Although the opposite situation was observed at the irrigation interval of 8 days, the effect was not statistically significant. Overall, I_1M_1 and I_1M_0 had the highest plant dry matter (23.46 and 22.7%, respectively), and I_3M_0 had the lowest plant dry matter (16.03%) (Table 4).

Shoot fresh weight decreased with a reduction of irrigation frequency. As seen in table 4, treatments I_4M_1 and I_4M_2 were not significantly different from each other, and they produced the lowest shoot dry weight of 5.526 and 5.806 g respectively. The highest shoot fresh weight of 8.636 g was recorded for plants treated with I_1M_0. The shoot dry matter percentage was significantly less in the 2-day irrigation interval compared with the 4, 6- and 8-day intervals over all three SA rates. The lowest shoot dry matter was observed in I_4M_0 (14.70%) and I_3M_2 (14.93%) and the highest shoot dry matter of 26.2% was observed in I_1M_0 (Table 4).

Table 1. Analysis of variance for the effects of different treatments on the morphological traits of *C. roseus*.

SoV	df	Flower number	Flower diameter	Plant height	Leaf number	Node number	Internode length	Leaf abscission	Plant fresh weight	Plant dry matter	Shoo fresh weight	Shootdry matter	Root fresh weight	Root dry matter	Root length
Irrigation interval (I)	3	207**	0.242**	3772**	340**	35.9**	9.80*	0.198^{ns}	13.6**	39.5**	9.002**	161.4**	0.549**	79.40**	385**
SA application (M)	2	4.65*	0.043**	1224**	67.6**	10.02**	14.7**	13.1**	2.10**	14.5**	2.292**	0.305^{ns}	0.239**	45.51**	201**
I × M	6	31.06**	0.017*	303**	14.3**	0.842**	8.76*	1.84**	0.137*	6.53**	0.126*	4.25**	0.112**	38.27**	67.9**
Error	24	1.24	0.0071	46.6	1.63	0.138	2.42	0.382	0.0609	0.463	0.0361	0.649	0.016	2.65	3.44
CV (%)		6.06	9.32	6.2	4.73	2.7	20.17	11.8	3.17	3.5	2.64	4.32	22.03	6.96	2.62

*,** and ^{ns} show significance at the 5, 1% probability levels and insignificant in the LSD test, respectively.

Table 2. Comparison of means for the effect of foliar application of salicylic acid on the morphological traits of *C. roseus*.

Treatments	Flower number	Flower diameter (mm)	Plant height (cm)	Leaf number	Node number	Inter node length (mm)	Leaf abscission	Plant fresh weight (g)	Plant dry matter (%)	Shoot fresh weight (g)	Shoot dry matter (%)	Root fresh weight (g)	Root dry matter (%)	Root length (mm)
M_0	17.70^{b}	0.844^{b}	120.7^{a}	29.41^{a}	14.08^{b}	8.79^{a}	6.38^{a}	8.120^{a}	18.84^{b}	7.639^{a}	18.51^{a}	0.479^{b}	24.95^{a}	75.08^{a}
M_1	18.50^{ab}	0.923^{a}	100.6^{c}	24.66^{c}	12.75^{c}	6.58^{b}	5.00^{b}	7.302^{c}	20.72^{a}	6.770^{c}	18.80^{a}	0.532^{b}	24.02^{a}	66.91^{c}
M_2	18.93^{a}	0.962^{a}	108.8^{b}	27.00^{b}	14.50^{a}	7.79^{ab}	4.33^{c}	7.869^{b}	18.79^{b}	7.123^{b}	18.55^{a}	0.746^{a}	21.21^{b}	70.33^{b}

*With similar letter(s) in each column were not significant at the 1 and 5% probability level according to the LSD test. M_0, M_1 and M_2: 0, 1 and 2 time foliar application of 200 mg L^{-1} salicylic acid respectively.

Table 3. Comparison of means for the effect of the irrigation intervals on the morphological traits of *C. roseus*.

Treatments	Flower number	Flower diameter (mm)	Plantheight (cm)	Leafnumber	Node-number	Inter node length (mm)	Leaf abscission	Plant fresh weight (g)	Plant dry matter (%)	Shoot fresh weight (g)	Shootdry matter (%)	Root fresh weight (g)	Rootdry matter (%)	Root length (mm)
I_1	22.33^{a}	1.105^{a}	131^{a}	31.44^{a}	15.88^{a}	8.24^{ab}	5.26^{a}	9.00^{a}	21.90^{a}	8.243^{a}	24.80^{a}	0.754^{a}	27.8^{a}	72.4^{b}
I_2	21.77^{a}	0.981^{b}	114^{b}	32.00^{a}	14.66^{b}	6.47^{c}	5.16^{a}	8.32^{b}	20.45^{b}	7.517^{b}	17.96^{b}	0.809^{a}	21.8^{b}	69.7^{c}
I_3	17.44^{b}	0.811^{c}	112^{b}	25.88^{b}	13.33^{c}	8.85^{a}	5.43^{a}	7.59^{c}	18.06^{c}	7.091^{c}	16.01^{c}	0.507^{b}	22.1^{b}	78.3^{a}
I_4	11.97^{c}	0.742^{c}	82.0^{c}	18.77^{c}	11.22^{d}	7.32^{bc}	5.08^{a}	6.12^{d}	17.38^{d}	5.857^{d}	15.72^{c}	0.272^{c}	21.6^{b}	62.5^{d}

* With similar letter(s) in each column were not significant at the 1 and 5% probability level according to the LSD test. I_1, I_2, I_3 and I_4: Irrigation intervals of 2, 4, 6 and 8 days, respectively.

Table 4. Comparison of means for the interaction of the irrigation intervals × foliar application of salicylic acid on the morphological traits on *C. roseus*.

Treatments	Flower number	Flower diameter (mm)	Plant height (cm)	Leaf number	Node number	Internode length (mm)	Leaf abscission	Plant fresh weight (g)	Plant dry matter (%)	Shoot fresh weight (g)	Shoot dry matter (%)	Root fresh weight (g)	Rootdry matter (%)	Root length (mm)
I_1M_0	23.66^{b}	1.054^{ab}	154^{a}	36.00^{a}	16.00^{b}	9.243^{a}	5.833^{b}	9.214^{ab}	22.70^{ab}	8.636^{a}	26.20^{a}	0.570^{bc}	24.76^{cde}	79.00^{b}
I_1M_1	22.00^{bc}	1.084^{a}	117^{bc}	28.66^{c}	15.00^{c}	7.853^{abc}	5.566^{bc}	8.420^{c}	23.46^{a}	7.783^{c}	24.10^{b}	0.637^{b}	28.73^{ab}	68.00^{ef}
I_1M_2	21.33^{cd}	1.176^{a}	121^{b}	29.66^{bc}	16.66^{a}	7.633^{abc}	4.400^{de}	9.366^{a}	19.53^{c}	8.310^{b}	24.10^{b}	1.056^{a}	30.03^{a}	70.33^{de}
I_2M_0	22.33^{bc}	0.915^{bc}	117^{bc}	31.33^{b}	14.33^{d}	8.537^{abc}	6.966^{a}	8.868^{b}	19.46^{c}	8.300^{b}	17.16^{cde}	0.568^{bcd}	22.13^{efg}	72.00^{d}
I_2M_1	17.33^{e}	0.906^{cd}	106de	29.66^{bc}	13.66^{e}	8.164^{abc}	4.733^{cde}	7.719^{de}	22.00^{b}	7.040^{ef}	18.46^{c}	0.679^{b}	23.46^{def}	66.00^{fg}
I_2M_2	25.66^{a}	1.124^{a}	120^{b}	35.00^{a}	16.00^{b}	8.433^{abc}	3.800^{e}	8.394^{c}	19.90^{c}	7.213^{de}	18.26^{cd}	1.181^{a}	27.20^{bc}	76.66^{bc}
I_3M_0	16.00^{ef}	0.772^{def}	112^{bcd}	29.00^{c}	14.00^{de}	9.150^{ab}	7.400^{a}	7.799^{d}	16.03^{e}	7.380^{d}	16.00^{efg}	0.419^{cde}	20.06^{gh}	83.33^{a}
I_3M_1	20.00^{d}	0.872^{cde}	107^{cde}	24.00^{d}	12.00^{f}	8.980^{ab}	4.466^{de}	7.346^{e}	20.43^{c}	6.730^{f}	17.10^{cde}	0.616^{bc}	21.13^{fg}	75.66^{c}
I_3M_2	16.33^{ef}	0.788^{cde}	118^{b}	24.66^{d}	14.00^{de}	8.440^{abc}	4.333^{de}	7.651^{de}	17.73^{d}	7.163^{de}	14.93^{g}	0.487^{bcd}	18.23^{hi}	76.00^{bc}
I_4M_0	8.83^{h}	0.636^{f}	99.0^{e}	21.33^{e}	12.00^{f}	8.250^{abc}	5.333^{bcd}	6.598^{f}	17.16^{de}	6.240^{g}	14.70^{g}	0.358^{def}	25.73^{cd}	60.66^{hi}
I_4M_1	14.66^{f}	0.831^{cde}	71.6^{f}	16.33^{g}	10.33^{h}	7.033^{bc}	5.133^{bcd}	5.724^{g}	17.00^{de}	5.526^{h}	15.56^{fg}	0.197^{f}	22.76^{ef}	63.33^{gh}
I_4M_2	12.41^{g}	0.761^{ef}	75.3^{f}	18.66^{f}	11.33^{g}	6.687^{c}	4.800^{cde}	6.066^{g}	18.00^{d}	5.806^{h}	16.90^{def}	0.260^{ef}	16.53^{i}	58.33^{i}

* With similar letter(s) in each column were not significant at the 1 and 5% probability level according to the LSD test. I_1, I_2, I_3 and I_4: Irrigation intervals of 2, 4, 6 and 8 days, respectively. M_0, M_1 and M_2: 0, 1 and 2 time foliar application of 200 mg L^{-1} salicylic acid, respectively.

At irrigation intervals of 2 and 4 days, the increase in the frequency of SA application from one to two times was accompanied by higher root fresh weight, but at the irrigation interval of 6 days, the opposite effect was observed so that the increase in the frequency of SA application from one to two times resulted in the loss of root fresh weight. At the irrigation interval of 8 days, a one-time application of SA reduced root fresh weight. The root dry matter percentage increased with the application of SA at irrigation intervals of 2 and 4 days, whereas the application of SA resulted in a decrease in root dry matter percentage at the irrigation interval of 8 days.

A decrease in root length was observed with the application of SA at irrigation intervals of 2 and 6 days. At an irrigation interval of 4 days, a one-time application of SA resulted in shorter root length compared with the control; however, its two-time application increased this trait compared with the control. In the irrigation interval of 8 days, a one-time application of SA increased root length, but its repeated application decreased root length compared with the control (Table 4).

Physiological traits

Analysis of variance showed that the interaction I × M was significant for all of the physiological traits studied (MDA, SOD, POD, proline, petal anthocyanin, chlorophyll a and b, and total chlorophyll) at the one percent probability level (Table 5).

Since the interaction was significant for all physiological traits, the effects of the individual treatments are summarized only in tables 6 and 7.

The application of SA improved chlorophyll a content at irrigation intervals of 6 and 8 days, but decreased it at the irrigation interval of 2 days. In the irrigation interval of 4 days, the two-time application of SA increased chlorophyll a content compared with the control and the one-time application. The salicylic acid application also enhanced chlorophyll b and total chlorophyll contents at irrigation intervals of 6 and 8 days, but a decrease was observed at the irrigation interval of 2 days compared with the control. Plants irrigated once every 4 days exhibited an increase in chlorophyll b and total chlorophyll when treated with SA twice compared with when they were not treated with SA or were treated once. Overall, I_1M_0 had the most favorable levels of chlorophyll b and total chlorophyll, and I_2M_1 was associated with the lowest chlorophyll b (1.733 mg g^{-1} FW) and total chlorophyll (5.143 mg g^{-1} FW; Table 8).

The petal anthocyanin level was highest in I_1M_0 (4.83 mg 100 g^{-1} FW) and I_1M_2 (4.8 mg 100 g^{-1} FW). Anthocyanin level decreased significantly with a decrease in irrigation frequency, and I_4M_0 exhibited the lowest petal anthocyanin content of 3.06 mg 100 g^{-1} FW. However, SA application increased the petal anthocyanin content at the irrigation interval of 8 days compared with the untreated plants (Table 8).

Comparison of the means for petal proline content as affected by the I × M interaction reveals that SA application improved this trait at irrigation intervals of 2 and 6 days. Also, at the irrigation interval of 4 days, plants treated with SA had higher proline content than those treated only with distilled water. At the irrigation interval of 8 days, there was no significant difference between treatment with distilled water and one-time application of SA (0.0276 µmol g^{-1} DW), but two-time application of SA increased proline content to 0.0296 µmol g^{-1} DW (Table 8).

The MDA content decreased by the SA application at irrigation intervals of 4, 6 and 8 days as compared with the controls (distilled water treatments). The highest concentration of MDA (2.67 nmol g^{-1} FW) was obtained in I_1M_2, which was not significantly different to that of I_3M_0 (2.66 nmol g^{-1} FW). The lowest MDA concentration was 1.42 nmol g^{-1} FW recorded in I_4M_1 (Table 8).

Table 5. Analysis of variance for the effect of different treatments on the physiological traits of *C. roseus*.

SoV	df	Chlorophyll a	Chlorophyll b	Total chlorophyll	Petal anthocyanin	Proline	MDA	SOD	POD
Irrigation interval (I)	3	0.649**	0.389**	1.852**	2.24**	0.00021**	11705**	800**	0.129**
SA application (M)	2	0.577**	0.011^{ns}	0.734**	0.120*	0.00036**	11635**	2965**	0.244**
I × M	6	0.453**	0.236**	1.32**	0.206**	0.00012**	11601**	525**	0.034**
Error	24	0.029	0.044	0.107	0.026	0.000001	0.3333	133	0.0008
CV (%)		4.12	9.5	5.15	3.96	3.31	2.85	16.75	11.39

*, ** and ^{ns} show significance at the 5 , 1% probability levels and insignificant in the LSD test, respectively.

Table 6. Comparison of means for the effect of foliar application of salicylic acid on the physiological traits of *C. roseus*.

Treatments	Chlorophyll a (mg g^{-1} FW)	Chlorophyll b (mg g^{-1} FW)	Total chlorophyll (mg g^{-1} FW)	Petal anthocyanin (µmol g^{-1} FW)	Proline (µmol g^{-1} DW)	MDA (nmol g^{-1} FW)	SOD (IU g^{-1} FW)	POD (nmol g^{-1} FW)
M_0	4.042^{b}	2.220^{a}	6.26^{b}	3.995^{b}	0.0249^{c}	2.44^{b}	86.12^{a}	0.107^{c}
M_1	3.998^{b}	2.185^{a}	6.18^{b}	4.041^{b}	0.0326^{b}	2.08^{b}	65.25^{b}	0.260^{b}
M_2	4.398^{a}	2.247^{a}	6.64^{a}	4.187^{a}	0.0355^{a}	56.19^{a}	55.32^{c}	0.392^{a}

* With similar letter(s) in each column were not significant at the 1 and 5% probability level according to the LSD test. M_0, M_1 and M_2: 0, 1 and 2 time foliar application of 200 mg L^{-1} salicylic acid, respectively. FW = fresh weight; DW = dry weight.

Table 7. Comparison of means for the effect of the irrigation intervals on the physiological traits of *C. roseus*.

Treatments	Chlorophyll a (mg g^{-1} FW)	Chlorophyll b (mg g^{-1} FW)	Total chlorophyll (mg g^{-1} FW)	Petal anthocyanin (µmol g^{-1} FW)	Proline (µmol g^{-1} DW)	MDA (nmol g^{-1} FW)	SOD (IU g^{-1} FW)	POD (nmol g^{-1} FW)
I_1	4.218^{b}	2.292^{a}	6.51^{b}	4.644^{a}	0.0274^{c}	2.48^{b}	63.53^{b}	0.133^{d}
I_2	3.885^{c}	1.915^{b}	5.80^{c}	4.283^{b}	0.0302^{b}	2.29^{bc}	69.70^{b}	0.173^{c}
I_3	4.491^{a}	2.391^{a}	6.88^{a}	3.883^{c}	0.0381^{a}	4.33^{a}	60.53^{b}	0.320^{b}
I_4	3.990^{c}	2.271^{a}	6.26^{b}	3.488^{d}	0.0283^{c}	1.86^{c}	81.83^{a}	0.386^{a}

* With similar letter(s) in each column were not significant at the 1 and 5% probability level according to the LSD test. I_1, I_2, I_3 and I_4: Irrigation intervals of 2, 4, 6 and 8 days, respectively. FW = fresh weight; DW = dry weight.

Table 8. Comparison of means for the interaction of the irrigation intervals × foliar application of salicylic acid for the physiological traits of *C. roseus*.

Treatments	Chlorophyll a (mg g^{-1} FW)	Chlorophyll b (mg g^{-1} FW)	Total chlorophyll (mg g^{-1} FW)	Petal anthocyanin (µmol g^{-1} FW)	Proline (µmol g^{-1} DW)	MDA (nmol g^{-1} FW)	SOD (IU g^{-1} FW)	POD (nmol g^{-1} FW)
I_1M_0	4.590^{ab}	2.693^{a}	7.283^{a}	4.83^{a}	0.0210^{h}	2.41^{b}	85.50^{a}	0.110^{gh}
I_1M_1	3.876^{d}	2.020^{def}	5.896^{de}	4.30^{b}	0.0253^{g}	2.36^{bc}	62.50^{bc}	0.120^{gh}
I_1M_2	4.190^{c}	2.163^{bcde}	6.353^{cd}	4.80^{a}	0.0360^{c}	2.67^{a}	42.60^{d}	0.170^{f}
I_2M_0	3.810^{d}	1.876^{ef}	5.686^{ef}	4.26^{b}	0.0176^{i}	2.45^{b}	83.30^{a}	0.080^{h}
I_2M_1	3.410^{e}	1.733^{f}	5.143^{f}	4.21^{bc}	0.0420^{b}	2.21^{bc}	79.60^{ab}	0.170^{f}
I_2M_2	4.436^{abc}	2.136^{cde}	6.573^{bc}	4.36^{b}	0.0310^{e}	2.20^{bc}	46.20^{cd}	0.270^{e}
I_3M_0	4.313^{bc}	2.316^{bcd}	6.630^{bc}	3.81^{de}	0.0333^{d}	2.66^{a}	88.00^{a}	0.130^{fg}
I_3M_1	4.466^{abc}	2.506^{ab}	6.973^{ab}	3.98^{cd}	0.0356^{c}	2.35^{bc}	46.60^{cd}	0.320^{d}
I_3M_2	4.693^{a}	2.350^{abcd}	7.043^{ab}	3.85^{de}	0.0453^{a}	2.18^{bc}	47.00^{cd}	0.510^{b}
I_4M_0	3.456^{e}	1.993^{def}	5.450^{ef}	3.06^{f}	0.0276^{f}	2.25^{bc}	87.70^{a}	0.100^{gh}
I_4M_1	4.240^{c}	2.480^{abc}	6.720^{bc}	3.80^{de}	0.0276^{f}	1.42^{c}	72.30^{ab}	0.440^{c}
I_4M_2	4.273^{c}	2.340^{abcd}	6.613^{bc}	3.60^{e}	0.0296^{e}	1.91^{bc}	85.50^{a}	0.620^{a}

* With similar letter(s) in each column were not significant at the 1 and 5% probability level according to the LSD test. I_1, I_2, I_3 and I_4: Irrigation intervals of 2, 4, 6 and 8 days, respectively. M_0, M_1 and M_2: 0, 1 and 2 time foliar application of 200 mg L^{-1} salicylic acid, respectively. FW = fresh weight; DW = dry weight.

As is evident in table 8, SOD declined with SA application at all irrigation intervals; however, the differences were not statistically significant in all treatments. The lowest SOD (42.6 IU g^{-1} FW) was obtained in I_1M_2. The highest SOD activities were observed in I_3M_0, I_4M_0, I_1M_0, I_4M_2, and I_2M_0 (Table 8), and these values did not differ significantly from one another. Peroxidase enzyme activity was increased with SA application at all irrigation intervals. The highest POD (0.62 nmol g^{-1} FW) was recorded in I_4M_2 and the lowest in I_2M_0 (0.08 nmol g^{-1} FW) (Table 8).

DISCUSSION

The present study focused on the effect of drought stress and its modification by the application of SA on morphological and physiological characteristics of *(C. roseus)*. The results showed that treatment I_1M_0 was superior in most of the traits measured. The application of SA, especially in I_2M_2, increased flower number per plant, flower diameter, the number of leaves per plant, root fresh weight and chlorophyll *a* content, and decreased leaf abscission. It has been shown that the SA application under water deficit stress, alleviates the adverse effects of stress and makes the plants more adaptable to drought stress (Kang and Wang, 2003), which supports our observations.

Amiri *et al*. (2016) reported that water deficit stress was related to the production of fewer branches and shorter plants, but that SA application improved these two traits significantly. Hussein *et al.* (2007) reported an increase in plant height, number of leaves, and root and stem dry weight of maize plants treated with SA.

Farooq *et al*. (2010) and Delany *et al*. (1994) suggest that SA application to plants exposed to environmental stresses can enhance their growth by improving carbon fixation, metabolite synthesis, retention of water status in plant tissues, and photosynthesis retention. In addition, it has been shown that the SA application to plants exposed to drought and salinity stresses can improve their resistance and growth (manifested in higher plant height, internode length and number, etc.)

through improving nutrient uptake (Eraslan *et al*., 2007; Du *et al*., 1998). Since we found that the treatment that had the highest root fresh weight was the most effective for reducing leaf abscission and increasing flower number and diameter as well as leaf number per plant, we can infer that the effect of SA on root growth also resulted in improved uptake of water and nutrients, resulting in the enhancement of morphological traits of *C. roseus*.

Dat *et al*. (1998) relate the resistance of plants treated with SA to the effect of this compound on the expression of genes responsible for plant protection against environmental stresses. A number of authors have proposed that SA regulates plant evolution by synthesizing protein kinases that are responsible for the division, regulation, and differentiation of cells, thereby playing an effective role in plant adaptation to stress and the maintenance of normal vegetative growth (Salarpour Ghoraba and Farahbakhsh, 2015; Eraslan *et al*., 2007; Hayat *et al*., 2010).

We found that SA application of irrigation intervals of 6 and 8 days was associated with an increased amount of leaf and petal pigments. Proline and peroxidase contents were increased with SA application at an irrigation interval of 4 days. Salicylic acid application resulted in a loss of SOD at all four irrigation intervals and a loss of MDA at irrigation intervals in 4, 6 and 8 days. The desirable effect of SA on reducing the effects of stress are related to a number of actions: its inhibition of the biosynthesis of ethylene; enhancement of photosynthesis and chlorophyll content; closure of stomata; and its effect on the activity of catalase, peroxidase and osmotic regulators such as proline, glycine, and betaine (Popova *et al*., 2013; Amiri *et al*., 2016). Amiri *et al*. (2016) found that low rates of irrigation reduced chlorophyll and proline, but the effect was offset by foliar application of SA. Singh and Usha (2003) named the loss of photosynthetic pigments as one of the most destructive effects of drought stress. In Gornik *et al*. (2008), the chlorophyll content of grapevines was decreased under drought stress conditions. Nematollahi *et al*. (2013) reported the loss of chlorophyll content in two sunflower cultivars under drought stress, but SA application improved chlorophyll content, which is in agreement with our study. Delany *et al*. (1994) argue that SA increases photosynthesis by increasing the amount of chlorophyll in the leaves, thereby improving growth of the plants by providing suitable nutritional conditions. The increase in leaf chlorophyll content with an SA applied under drought stress has been reported in several other studies (Korkmaz *et al*., 2007; Khodary, 2004) which is consistent with our results.

EL-Tayeb (2005) argues that the maintenance of chlorophylls under stress condition results in photosynthetic stability and thus reduces the damage of environmental stresses. The increase in photosynthetic pigments, proline, and sugars and the decrease in the peroxidation of membrane lipids are all indicative of the alleviation of oxidative damage and the role of SA in increasing stress tolerance (Keshavarz *et al*., 2012). EL-Tayeb (2005) reported that foliar application of SA enhanced chlorophyll and carotenoid contents and that the favorable effect of SA on the retention of pigments was related to its effect on the rate of photosynthesis.

Shakirova *et al*. (2003) found that SA protects the membranes and cell organelles against degradation by reducing oxidative stresses and increasing proline content. These authors also reported that foliar application of SA reduces the extent of the peroxidation of lipids and hydrogen peroxide and protects the photosynthetic structure, thereby inhibiting the destruction of pigments, especially chlorophylls Qinghua and Zhujun (2008) reported the reduction of MDA generation in *Lemna* under environmental stresses due to SA application, which agrees with the results of the present study.

Salarpour Ghoraba and Farahbakhsh (2015) reported that dehydration, increased MDA in many plants, but foliar application of SA reduced MDA in fennel. Katsuhara *et al*. (2005) suggest that the increase in MDA under stress conditions is due to the damage to lipids and membrane fatty acids by free radicals. , In the present study, SA reduced MDA by reducing the destructive effects of water scarcity.

CONCLUSION

Flower number, flower diameter, plant height, and flower and leaf pigments are the most important parameters for flower-bearing plants like *Catharanthus roseus*. We found that the experimental plants exhibited their best morphological and physiological traits at irrigation intervals of 2 and 4 days. Therefore, we conclude that an irrigation interval of 4 days can be effective to promote growth and optimum traits in *C. roseus*. At irrigation intervals of 6 and 8 days, which imposed drought stress on the plants, SA application could improve the traits. Further studies are required for the measurement of alkaloids under water deficit conditions and SA application in order to accomplish more coherent results.

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