Journal of Ornamental Plants
Available online on: www.jornamental.iaurasht.ac.ir
ISSN (Print): 2251-6433 ISSN (Online): 2251-6441
Research Paper

Alleviation of Drought Stress Effects by Exogenous Application of Spermidine and Salicylic Acid on Hollyhock (*Alcea rosea*)

Toktam Oraee, Mahmoud Shoor*, Ali Tehranifar and Hossein Nemati

Department of Horticultural Science and Landscape Faculty of Agriculture, Ferdowsi University of Mashhad

Received: 07 November 2018 Accepted: 13 August 2019
*Corresponding author's email: shoor@um.ac.ir

Abstract

Drought is one of the most important environmental factors limiting the cultivation of ornamental plants in green spaces. The effects of spermidine and salicylic acid were investigated on hollyhock (*Alcea rosea* L.) under drought stress. At first, the plants were sprayed with three doses of spermidine and three doses of salicylic acid (control, 100, 200 and 400 µM) for three consecutive days and they were then subjected to drought stress (40, 60, 80 % FC) for two weeks. The results showed that the increase in drought stress up to 40% FC increased electrolyte leakage, proline, and superoxide dismutase enzyme activity compared to the control plants. Also, the application of 100 µM spermidine and salicylic acid at different concentrations of spraying solutions significantly reduced electrolyte leakage and catalase enzyme activity and increased relative water content (RWC), proline, protein, number of flowers, leaf area, and superoxide dismutase enzyme activity, but the higher concentration (400 µM) was ineffective or had inhibitory effects. The plants treated with 100 µM spermidine and salicylic acid showed higher tolerance to drought stress (up to 40% FC) with regard to lower electrolyte leakage (by 5%) and higher relative water content (by 11 and 9%), proline content (by 31 and 21%), SPAD (by 18 and 5%), and dry weight (by 3%) compared with the non-treated plants under 40% FC. Hollyhock growth was severely damaged by water deficit, but the application of spermidine and salicylic acid promoted RWC, proline, and protein content under water deficit conditions. Foliar application of spermidine and salicylic acid can be considered an economical practice to increase hollyhock performance under water deficit conditions.

Keywords: Antioxidant activity, Foliar spray, Hollyhock, Stress.

INTRODUCTION

Considering the increasing population growth throughout the world, green space sustainability has become highlighted. The intensification of environmental stresses has led to the destruction of ornamental plants. Thus, one of the primary objectives of plant producers and modifiers is to provide high-quality plants that are resistant to environmental stresses in order for them to be properly utilized in green spaces (Noman *et al*., 2017). Obviously, to determine plant species that will be cultivated in green spaces, decisions are made based on plant resistance to unfavorable factors and water quantity. Due to bearing flowering branches in spring and summer, hollyhock plants are used in green space designs. Moreover, this plant is of high importance due to its long flowering period and resistance to chilling stress. Hollyhock (*Alcea rosea* L.) is a perennial plant native to East Asia that includes 70 species out of which 36 grow in Iran (Pakravan and Ghahreman, 2003). The plant is highly paid attention due to its pigments and colored substances. The flowers of this plant are also used to produce a kind of tea. On the other hand, its antibacterial (Ahmed *et al*., 2016) and antioxidant (Hussain *et al*., 2014) properties have been scientifically proven.

Drought stress is one of the most important stresses affecting plant yields (Ghodke *et al*., 2018). Plants have evolved various drought resistance strategies at the morphological and physio-biochemical levels to respond and adapt to drought stress (Larkunthod *et al*., 2018; Almeida *et al*., 2019). Plants' drought resistance can be classified into four major mechanisms: drought escape, drought avoidance, drought tolerance, and drought recovery, among which drought avoidance via enhanced RWC and drought tolerance via osmotic adjustment and antioxidant defense system are the two major mechanisms (Farooq *et al*., 2009b; Krasensky and Jonak, 2012; Fang and Xiong, 2015).

Cell membranes are the first main spot in the cell that are exposed to stress-induced damages, and increasing the percentage of electrolyte leakage leads to cell death (Barranco *et al*., 2005). The use of polyamines increases the stability of cell membranes (Khaleghi *et al*., 2016). On the other hand, by entering chloroplasts, they protect the photosynthesis system against the harmful effects of environmental stresses (Adam and Murthy, 2013). In a research study, Zhang *et al*. (2010) observed that the moderating effect of spermidine on photosynthesis was stronger in drought-sensitive cultivars than in drought-resistant ones.

Environmental and non-environmental stresses lead to the formation of reactive oxygen species (ROS). ROS causes peroxidation of membrane lipids as well as the destruction of proteins and nucleic acids (Jiang and Zhang, 2001). ROS's are controlled through enzymatic or non-enzymatic antioxidant defense system. Superoxide dismutase can be mentioned as one of the enzymatic antioxidants (Alscher *et al*., 2002; Agarwal and Pandey, 2004). Several studies have reported changes in the activity of many enzymes of the antioxidant defense system under different environmental stress (Faize *et al*., 2011; Tyagia *et al*., 2017; Guo *et al*., 2018). Moreover, the destruction of proteins and the accumulation of certain free amino acids to maintain and adjust the osmotic pressure of cells and reduce the protein synthesis have been observed under stress conditions (Michaletti *et al*., 2018; Zhang *et al*., 2018).

This species is an annual plant that can be easily cultivated from seeds. The plant loves warm weather and sunlight. Although hollyhock can be easily grown in a warm climate, field production is restricted by environmental factors and management practices. For instance, limited water availability is a common production limitation. Therefore, supplemental irrigation is necessary for commercial production of hollyhock. Even with some water availability, traditional practices of growing plants fall short in supporting commercial production. It will be very useful for plants to find ways to improve their tolerance to this stress. The use of plant growth regulators (PGRs) is a possible approach for increasing plant tolerance to stresses among other methods, such as breeding and genetic engineering. Thus, in addition to the function of drought stress-resistant

plants, the external function of some polyamines such as putrescine, spermine and spermidine increases the resistance of plants (Hayat *et al*., 2008; Koyro *et al*., 2012).

Furthermore, salicylic acid plays a key role in improving the resistance of plants to drought stress and influences indices such as photosynthesis, hydrologic relationships, and carbohydrate and proline contents (Fariduddin *et al*., 2003). An experiment was conducted to clarify the external function of salicylic acid in the antioxidant activity of carrot plants under stress. The results showed that the use of this regulator increased the total antioxidant activity in roots and branches and accumulated proline in this plant (Eraslan *et al*., 2007). However, research on the impact of spermidine (Spd) and salicylic acid (SA) foliar application on drought tolerance of hollyhock is scarce. Therefore, the present study provides novel evidence concerning a less studied ornamental and medical plant like hollyhock, contributing to a useful complementary assessment of significant causal relationships among the studied variables under different conditions. In this regard, the study adds to a better understanding of Spd and SA effects on hollyhock response to water deficit.

MATERIALS AND METHODS

This experiment was conducted at the Campus Faculty of the Department of Horticulture at Ferdowsi University, Mashhad during 2015-2016 as a factorial experiment based on a complete randomized design with two factors, including foliar spraying and drought stress, and with three replicates (3 pots per replicate). The treatments consisted of three concentrations of spermidine (Spd) and three concentration of salicylic acid (SA) (100, 200 and 400 μM) and non-sprayed plants as control, as well as three irrigation levels, 80% FC as control, 60% FC (moderate deficit irrigation) and 40% FC (severe deficit irrigation).

Hollyhock (*Alcea rosea* L.) seeds were provided by the Iranian Biological Resource Center (IBRC). The seeds were sown in trays with cocopeat and perlite mix in July. After the germination period, plants were watered three times per week and fertilized every week with full-strength Hoagland solution (Hoagland and Arnon, 1950). In October (5-leaf stage), the plants (one plant per pot) were planted in pots (18 cm height and 8 cm diameter) filled with a mixture of garden soil, sand and rotted manure (2:1:1) in a greenhouse in February. In order to determine the effect of foliar spray with Spd and SA on drought stress, after the emergence of the first flower in May, water stress treatments were applied as follows: irrigation to meet 80, 60 and 40% of FC for two weeks by Time Domain Reflectometry (TDR). The amount of water requirement for each plant was determined at each treatment according to the following formula:

$$Vw= \{(FC - \theta)(Bd \times D \times A)\}$$

where FC is the field capacity, θ is the gravimetric water content (, m is the wet soil volume, V is the dry soil volume, D is the depth of root development, Bd is the bulk density of soil, and *A* is the pot area (Ahmadiyan *et al*., 2010)

At the end of the experiment, chlorophyll meter (SPAD) readings were recorded per leaves of each plant and the mean reading was noted. Data on the number of flowers were recorded. Leaf area was determined by a leaf area meter (LI-3000 C). To measure electrolyte leakage, proline, relative water content (RWC), protein, superoxide dismutase, and catalase activity, the top leaf tissues were collected from a minimum of three independent plants. To determine dry matter content, the harvested parts of the plant (stem, leaves, and shoots) were oven-dried at 70°C for 72 h.

Electrolyte leakage assay

After drought stress, a disc with 8 mm diameter of leaf was removed and placed in tubes

containing 40 ml distilled deionized water. Electrical conductivity (EC) of the leaf was measured on the following day using a solution analyzer (Cole-Parmer Instrument Co., Chicago). To determine potential EC_1, the samples were then autoclaved at 121°C for 20 min to release the total electrolytes from the samples. After maintaining the samples at 21°C overnight, EC_2 of the leachate was measured. The percentage of EL for each plant was determined at each treatment temperature according to the following formula (Reddy *et al*., 2004):

$$EL\ (\%) = EC_1 / EC_2 \times 100$$

Proline assay

Leaves were harvested, submerged in liquid nitrogen, and stored at -80°C to measure their proline content according to the method of Bates *et al*. (1973). A sample weighing 300 mg was homogenized in 3% sulfosalicylic acid and filtered. Then, 2 mL of the extracted solution was combined with 2 mL of acid-ninhydrin and 2 mL of glacial acetic acid and heated in a water bath at 100°C for 1 h. The reaction was terminated in an ice bath and then, 4 mL of toluene was added to the mixture. After the phase separation, the upper chromophore containing toluene was transferred to a cuvette and measured at 520 nm in a spectrophotometer. The proline concentration was determined based on a standard curve and expressed in μmol^{-1} FW.

Relative water content (RWC) assay

Relative water content was determined using the method described by Turner (1981). RWC was calculated based on the following formulae:

$$RWC\ (\%) = ((FW-DW))/((TW-DW))\times 100$$

where FW is the fresh weight, DW is the dry weight, and TW is the turgor weight of the leaf samples.

Soluble protein and antioxidant enzyme extraction

Approximately 300 mg of leaf and crown tissues (fresh weight) were harvested, frozen in liquid nitrogen, and stored at -80°C for the determination of soluble protein and antioxidant enzyme activities. Extraction was based on methods previously described by DaCosta and Huang (2007). Tissues were homogenized in 4 mL of 150 mM cold phosphate buffer (pH 7.0) and centrifuged at 12,000 rpm for 30 min at 4°C. The supernatant was transferred to 15-mL tubes and used for soluble protein and enzyme activity determination.

Total soluble protein assay

Soluble protein determination was based on the Bradford assay (1976). A 3-mL aliquot of a solution containing Coomassie blue G-250 and 95% ethanol was added to a tube containing 100 L of protein extract. The samples were vortexed and absorbances were read at 595 nm following a 5-min color development period. Protein concentration was determined using bovine serum albumin as a standard and expressed in mg ml^{-1}.

Antioxidant enzyme activity assay

Catalase (CAT) activity was determined using the method of Chance and Maehly (1955) with some modifications. The oxidation of H_2O_2 was initiated by adding a 100-L aliquot of the extract to a solution containing 50 mM phosphate buffer (pH 7.0) and 45 mM H_2O_2. The enzyme activity was determined by measuring changes in absorbance every 10 s for 60 s using a spec-

trophotometer. One unit of CAT activity was defined as the change in absorbance of 0.01 per min and expressed in units per milligram soluble protein basis. Superoxide dismutase (SOD) activity was determined using the method of Giannopolitis and Reis (1977) with some modifications. A 100-L aliquot of the extract was added to a solution containing 50 mM phosphate buffer (pH 7.8), 60 M riboflavin, 195 mM methionine, 3 M EDTA, and 1.125 mM nitro blue tetrazolium (NBT) ditetrazolium chloride. A solution using no enzyme extract was used as a control. The test tubes were irradiated under fluorescent lights at approximately 300 μmol $m^{-2}s^{-1}$ for 30 min, and they were then placed in the darkness for 10 min to stop the reaction. The absorbance was measured at 560 nm and one unit of enzyme activity was defined as the amount of enzyme that would inhibit 50% of NBT photoreduction and expressed in units per milligram soluble protein basis.

Data were arranged in a two-factor factorial experiment based on a completely randomized design and analyzed using SAS JMP 13.2 software. Differences in means were compared by the least significant difference (LSD) test at the $P < 0.05$ level.

RESULTS AND DISCUSSION

Electrolyte leakage was significantly affected by drought ($P<0.01$), Spd and SA concentrations ($P<0.01$) and their interaction ($P<0.05$). Drought stress significantly increased electrolyte leakage by 5.2% and the application of 100 and 200 μM Spd and SA decreased electrolyte leakage of the plants (Table 1).

The highest electrolyte leakage was recorded in the plants grown under moderate and severe deficit irrigation (60 and 40% FC) and sprayed by 400 μM and the lowest was observed in the plants sprayed by 100 μM Spd and SA and grown under 80% FC (Fig. 1a). The increase in electrolyte leakage in plants under drought stress has been reported and verified by many research works (Rezayian *et al*., 2018; Mervad *et al*., 2018). Plant treatment with the mentioned compounds increased membrane stability and decreased ion leakage. It seems that higher concentrations of Spd and SA in all drought treatments have had an inhibiting role in membrane stability. Polyamines contribute to membrane stability and reduce oxidative stresses. Their utilization increases the stability and integration of cell membranes in stressed plants (Gill and Tuteja, 2010). By activating the antioxidant system, SA treatment reduces free radicals and membrane protection against lipid peroxidation (Horvath *et al*., 2007) which has, in turn, led to an ionic reduction in the recent experiment.

In general, 40% FC treatment resulted in the reduction of SPAD compared to 80 and 60% FC treatments (by 9% and 6%, respectively). Also, SPAD was significantly ($P<0.01$) increased in all foliar spraying compared to the control (Table 1). SPAD was significantly affected by the interactive effects of drought stress and foliar spraying ($P<0.01$). In all the irrigation treatments, SPAD was constant in 100 and 200 μM Spd and SA, but it was decreased in 1000 μM SA. Based on Fig. 1b, the plants exhibited higher and lower SPAD in response to 100 μM Spd under 80% FC and 400 μM SA under 40% FC, respectively. The reduction in chlorophyll content under drought stress pertains to photo-oxidation of chlorophyll by active oxygen species (ROS), the increment of chlorophyllas enzyme activity, retransfer of nitrogen from leaves, and reduction in the absorption of nutrients. Spraying rapeseed with low concentrations of SA increases chlorophyll content, net photosynthesis, and carboxylation efficiency; however, higher concentrations decrease them (Fariduddin *et al*., 2003), supporting our results.

Drought stress, Spd, and SA concentrations had significant ($P<0.01$) effects on relative water content. RWC was decreased from 81% in the control to 78% in 40% FC. RWC reached its highest concentration (83%) in response to 100 μM Spd compared to all other treatments. The results showed that RWC was significantly increased by the application of 100 and 200 μM Spd and SA under drought stress conditions. RWC reached a peak when the plants were sprayed with 100

Table 1. The effect of drought stress and application of foliar spray on physiological and morphological characteristics of hollyhock plants.

Treatments		Electrolyte leakage (%)	SPAD	Relative water content (%)	Proline (µmol g^{-1} FW)	Protein (mg ml^{-1})	Superoxide dismutase (U mg^{-1} protein)	Catalase (U mg^{-1} protein)	Number of flower	Leaf area (cm^2)	Dry weight (g)
Drought stress (FC)	80%	24.8^{c}	55.1^{a}	80.7^{a}	0.509^{c}	2.77^{a}	22.1^{c}	36.7^{a}	12.2^{a}	2859^{a}	79.1^{a}
	60%	25.4^{b}	53.1^{b}	79.3^{b}	0.551^{b}	2.75^{b}	22.5^{b}	36.7^{a}	11.1^{b}	2829^{ab}	78.5^{b}
	40%	26.1^{a}	50.3^{c}	77.9^{c}	0.594^{a}	2.64^{c}	23.3^{a}	36.9^{a}	10.8^{b}	2809^{b}	76.8^{c}
Foliar spraying (µM)	Control	26.4^{b}	50.8^{e}	75.2^{e}	0.412^{d}	2.70^{c}	21.9^{e}	2.31^{a}	11.1^{c}	2740^{d}	77.5^{c}
	100 Spd	24.3^{e}	57.1^{a}	82.7^{a}	0.622^{a}	2.77^{a}	23.6^{a}	4.81^{b}	12.8^{a}	3110^{a}	81.3^{a}
	200 Spd	24.7^{cd}	53.5^{c}	82.1^{b}	0.591^{b}	2.73^{b}	23.1^{ab}	5.34^{a}	11.9^{bc}	2927^{b}	79.4^{b}
	400 Spd	26.6^{ab}	51.8^{d}	77.1^{d}	0.545^{c}	2.70^{c}	21.7^{c}	5.32^{a}	10.2^{d}	2583^{e}	74.6^{d}
	100 SA	24.5^{de}	54.7^{b}	82.1^{b}	0.594^{b}	2.78^{a}	23.4^{a}	4.74^{b}	12.1^{ab}	3077^{a}	81.1^{a}
	200 SA	24.8^{c}	52.2^{d}	81.2^{c}	0.575^{b}	2.72^{bc}	22.7^{b}	5.55^{a}	11.4^{bc}	2842^{c}	78.8^{b}
	400 SA	26.8^{a}	49.5^{f}	74.9^{e}	0.523^{c}	2.65^{d}	21.6^{e}	5.70^{a}	10.0^{d}	2549^{e}	74.5^{d}

*In each column, means with the similar letter(s) are not significantly different ($P < 0.05$) using the LSD test.

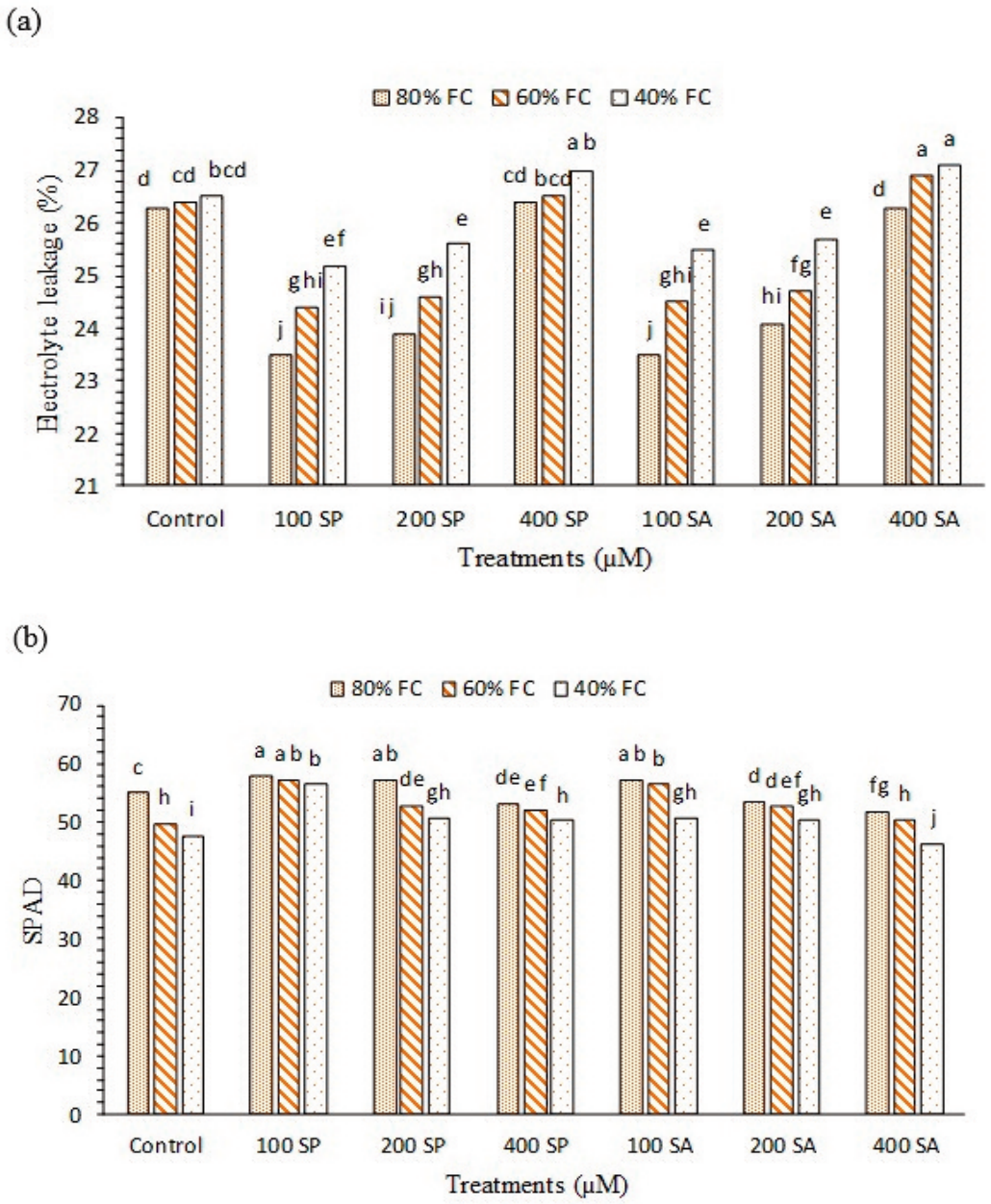

Fig. 1. The interactive effect of drought stress and foliar spray treatments on electrolyte leakage (a) and SPAD (b) in hollyhock. Data followed by different letter(s) above the bars indicate a significant difference at $P < 0.05$. (SP: Spermidine and SA: Salicylic acid).

µM Spd under 80 % FC (Table 1). No significant differences were observed in 400 µM SA treatment when the plants were exposed to drought stress compared to control (Fig. 2a). However, the exogenous application of SA has reportedly increased RWC of *Dendranthema grandiflorum* (Vahdati Mashhadian *et al*., 2012) and *Cyclamen persicum* (Farjadi Shakib *et al*., 2012). Mustafavi *et al*. (2015) reported the lack of any significant differences between Spd-treated plants and controls in valerian (*Valeriana officinalis* L.) plants under drought stress. In our experiment, the lack of significant difference in RWC between the SA-treated plants and the control showed that these treatments at high concentrations failed to reduce the negative effects of drought stress on RWC of the plants.

Drought stress and foliar spraying with Spd and SA also caused a significant ($P<0.01$) increase in proline content (Table 1). Plants exhibited higher proline in response to Spd and SA treatments compared to control. The maximum (0.613 µmol g^{-1} FW) and minimum (0.412 µmol g^{-1} FW) proline concentrations were observed in 100 µM Spd and the control, respectively (Table 1). After all drought treatments, the proline content in the plants with foliar spraying was largely increased and was significantly higher than that in the plants without foliar spraying (Fig. 2b).

As an osmotic index, proline increases the ability of roots to absorb water, helps maintain the turgor of cells, improves stomatal conductance, prevents membrane destruction, and eliminates free radicals under drought stress (Turner, 2018). Oraee *et al*. (2018) showed that in viola (*Viola*× *wittrockiana*), RWC at 60% and 40% FC was reduced by 6% and 24% versus 80% FC, respectively. The results of means comparison showed that foliar treatment of plants under drought stress with different concentrations of Spd and SA increases proline compared to that in controls. It was reported for rapeseed that drought stress increased the proline level, and the utilization of SA and putrescine significantly increased proline content in both under-stress plants and controls (Ullah *et al*., 2012).

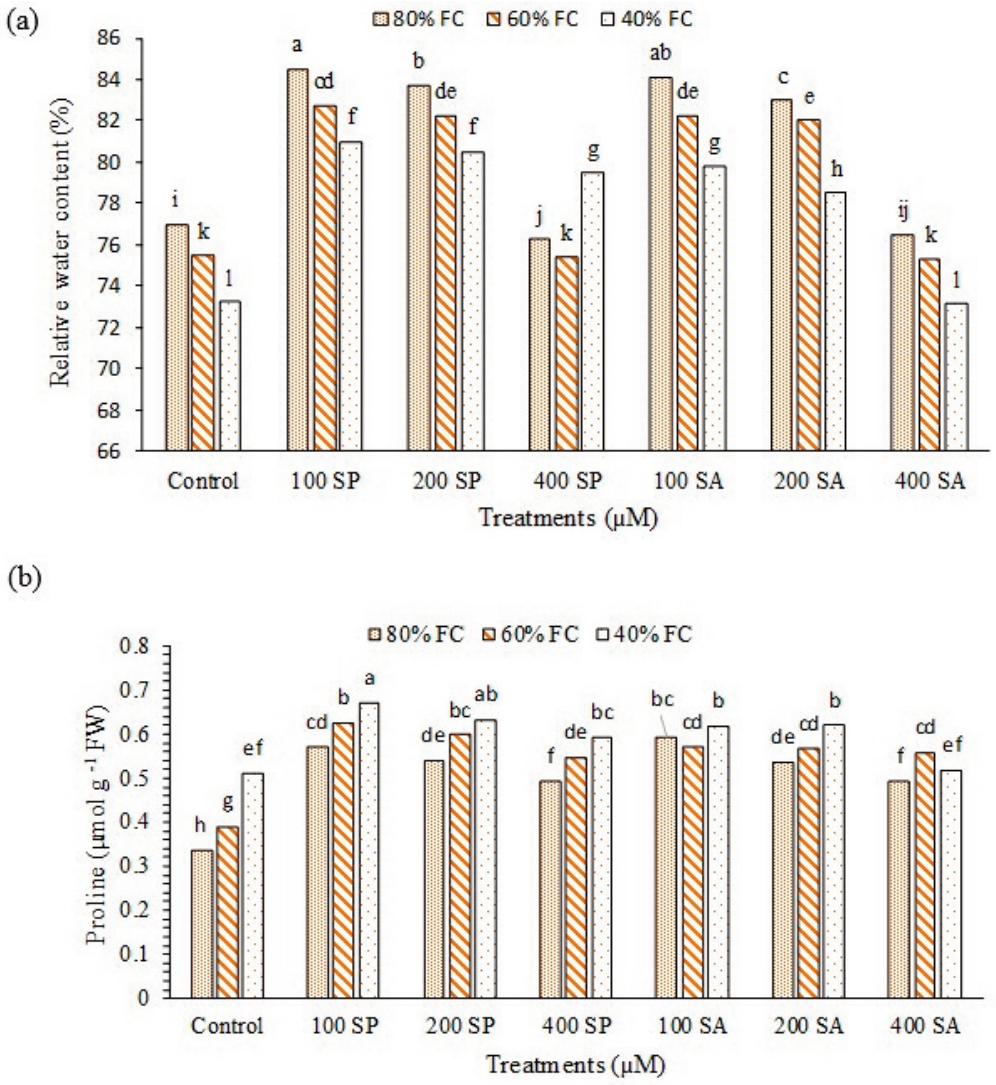

Fig. 2. The interactive effects of drought stress and foliar spray treatments on relative water content (a) and proline (b) in hollyhock. Data followed by different letter(s) above the bars indicate a significant difference at $P < 0.05$. (SP: Spermidine and SA: Salicylic acid).

It seems that SA raises proline metabolism, consequently the proline accumulation. As a signaling agent, it increases the expression of genes related to stress resistance (Agnes *et al*., 2005).

The total protein was significantly ($P<0.01$) increased in 80% FC compared to 60% and 40% FC. The maximum (2.77 mg ml^{-1}) and minimum (2.65 mg ml^{-1}) amount of protein were found in 100 µM Spd and 400 µM SA, respectively (Table 1). This index in plants sprayed with 100 and 200 µM Spd and SA under 80% and 60% FC conditions was increased compared to the control and no significant difference was observed between different concentrations compared to the control under 40% FC (Fig. 3a). Many researchers have reported an increase in protein content in plants exogenously applied with SA under drought stress (El Tayeb and Ahmad, 2010; Sawheny and Singh, 2002). Kang *et al*. (2005) identified 35 essential proteins that affected the induction of stress resistance along with SA. In an experiment, Farjadi Shakib *et al*. (2012) showed that the application of 1.5 mM SA increased the fresh weight of cyclamen compared to the control plants.

Superoxide dismutase activity was significantly affected by drought ($P<0.01$), foliar spraying ($P<0.01$) and their interaction ($P<0.05$). The plants exhibited higher superoxide dismutase activity in response to 40% FC treatment compared to 80% FC. As well, its activity was significantly increased (by 8%) by the application of 100 µM Spd and SA compared to the control (Table 1). Foliar spraying with all concentrations of Spd significantly increased superoxide dismutase activity under 80% FC compared to the control, but under 60% FC treatment, 100 and 200 µM Spd and 100 µM SA increased superoxide dismutase activity. There were no differences in response to all concentrations of foliar spray in 40% FC treatments compared to the control, except for the plants sprayed with 400 µM SA (Fig. 3b).

By stimulating different antioxidant systems, polyamines enhance stress resistance in plants. Additionally, the maximum activity of the superoxide dismutase enzyme in rice was achieved by applying 1 mM SA after 12 days of drought stress (Farooq *et al*., 2010). Nonetheless, the external application of SA does not always increase stress resistance. This depends on not only concentration and method of application but also the plant status including its development stage (Pan *et al*., 2006). This confirms the reduction of enzyme activity in higher concentrations of SA.

Although catalase activity was not affected by drought stress, lower concentrations of foliar spray significantly ($P<0.01$) decreased catalase activity and 100 µM Spd and SA resulted in 9.4 and 11.3% reduction in catalase activity, respectively, as compared to the control, but no difference was seen in response to other concentrations (Table 1.). Once the concentration of internal SA rises above a certain limit, it binds to catalase enzymes, inhibits their activity, and increases hydrogen peroxide level. This, in turn, provides a context for instigating the acquired resistance process (Horvath *et al*., 2007).

The number of flowers was significantly decreased by 11% in 40% FC compared to 80% FC. Also, the number of flowers was significantly increased by 15% and 10%, respectively, when the plants were sprayed with 100 µM Spd and 100 µM SA compared to the non-treated plants (Table 1). Table 1 shows that leaf area was dramatically decreased as a consequence of both water stress conditions, and the lowest amount was recorded at 40% FC. Flowering is another important parameter that is directly related to the yield and productivity of plants. SA has been reported to induce flowering in a number of plants, including Lemna (Cleland and Ajami, 1974). Different plant species including the ornamental plant *Sinningia speciosa* flowered much earlier as compared to the untreated control when they received an exogenous foliar spray of SA (Martin-Mex *et al*., 2003, 2005). SA and its close analogues enhanced leaf area and dry mass production in corn and soybean (Khan *et al*., 2003).

Dry weight was significantly affected by foliar spray, drought and their interaction ($P<0.01$). The response of 80% FC treatment compared to other irrigation treatments was better, and the highest dry weight (79.2 g) was assigned to this treatment. The dry weight of plants was

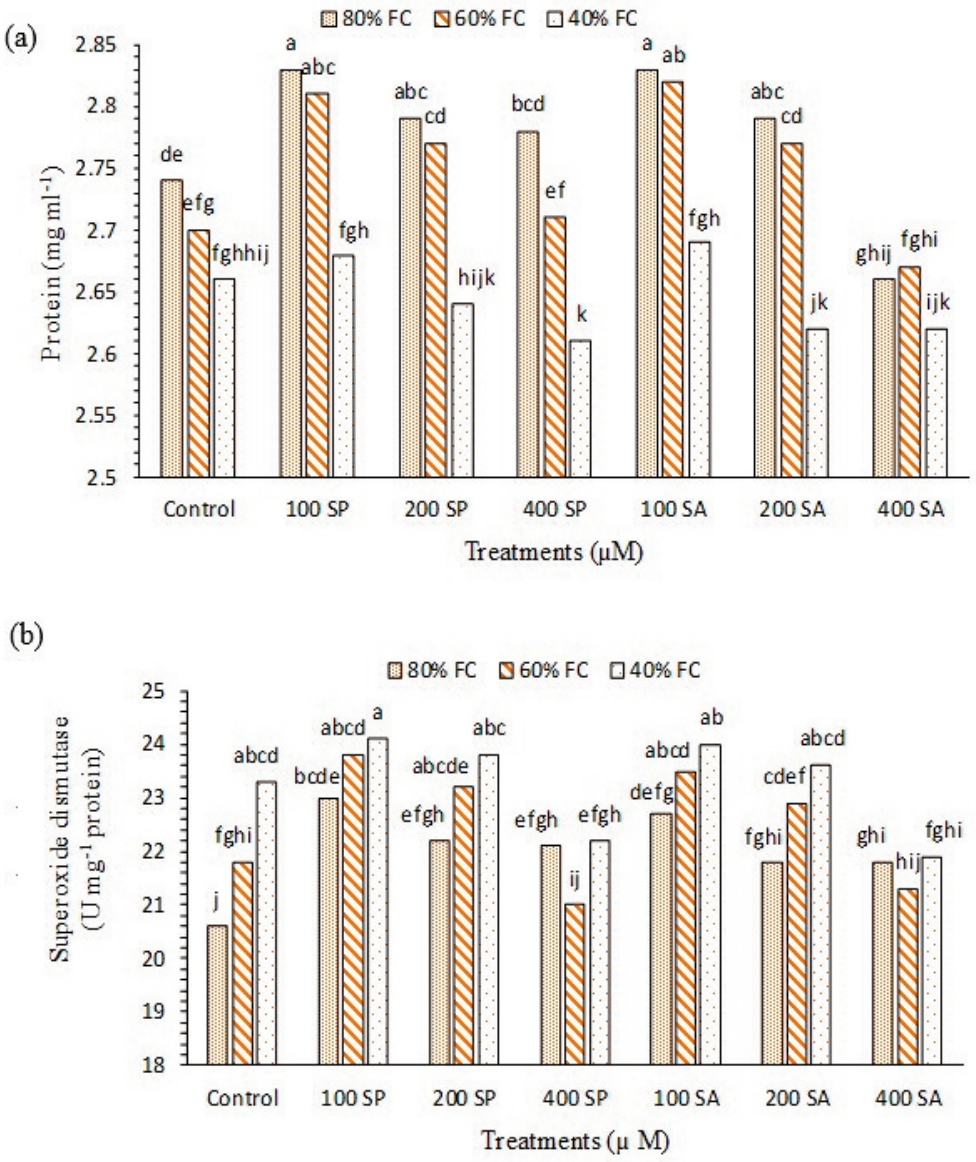

Fig. 3. The interactive effects of drought stress and foliar spray treatments on protein (a) and superoxide dismutase (b) in hollyhock. Data followed by different letter(s) above the bars indicate a significant difference at $P < 0.05$. (SP: Spermidine and SA: Salicylic acid).

significantly increased by 5 and 2%, respectively, when the plants were exposed to 100 µM Spd and SA compared to control (Table 1). The results showed that the dry weight was significantly increased by lower concentrations of foliar spray under drought conditions. The dry weight was the highest (82 g) when the plants were exposed to 100 µM Spd and SA under 80 and 60% FC conditions. The dry weights of plants showed a 4% reduction in 400 µM Spd and SA under 80, 60 and 40% FC (Fig. 4).

As drought stress increases, the dry weight of plants is reduced. The application of 2 µM SA increased the dry weight of petunia plants by 61%. In hollyhock plants, spraying 100 µM improved resistance to drought stress by increasing the dry weight so that the ability of SA to increase the plant dry weight and decrease the side effects of drought stress has significant impacts on improving the plant growth and overcoming the yield reduction in case of no irrigation treatment (Zarghami *et al*., 2014).

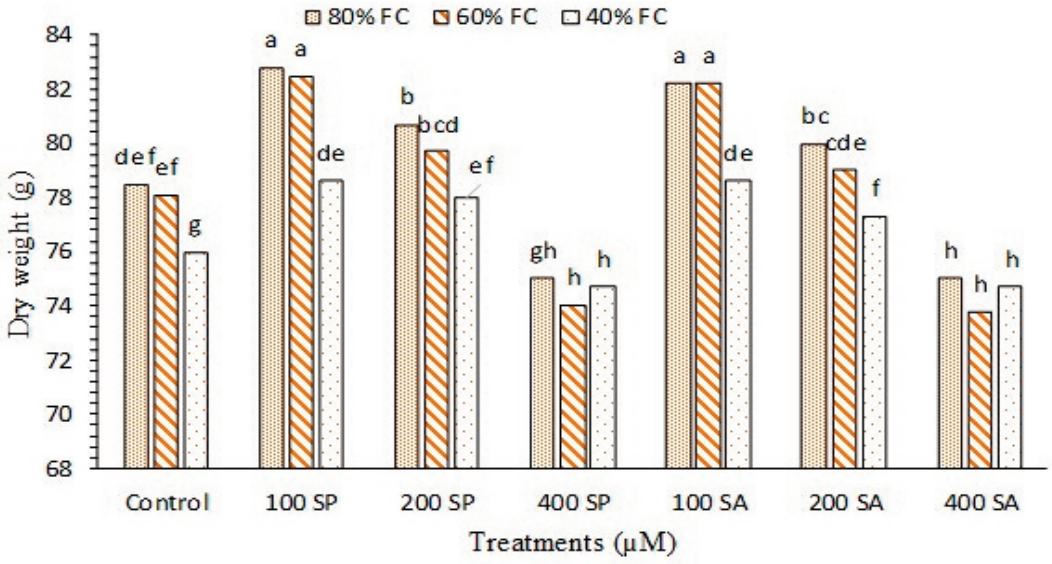

Fig. 4. The interactive effects of drought stress and foliar spray treatments on dry weight of hollyhock. Data followed by different letter(s) above the bars indicate a significant difference at $P < 0.05$. (SP: spermidine and SA: salicylic acid).

There was a significant negative relationship between electrolyte leakage (%) and other measured parameters. RWC showed the highest positive correlation ($r = 0.83^{**}$) with dry weight. The leaf chlorophyll content and the catalase activity had the highest negative correlation ($r = -0.38^{**}$) so that an increase in catalase activity resulted in the reduction of SPAD (Table 2). In the present experiment, the results of the correlation between traits indicated that the electrolyte leakage of the plants was increased under drought stress or higher SA concentration. The total protein and dry weight of the plants were significantly decreased while the electrolyte leakage was increased. The highest electrolyte leakage was recorded in the plants grown under 40% FC and sprayed by 400 µM Spd and SA and the lowest protein and dry weight were observed in the plants under these treatments.

Electrolyte leakage is a hallmark of stress response in intact plant cells. The electrolyte leakage is ubiquitous among different species, tissues, and cell types and can be triggered by all major stress factors. There are also some reports indicating that electrolyte leakage is used as a test for stress-induced injuries to plant tissues and 'a measure' of plant stress tolerance (Bajji *et al.*, 2002; Lee and Zhu, 2010).

There was a negative correlation between electrolyte leakage and CAT activity. A similar report by Farooq *et al*. (2009a) showed negative correlations between antioxidants and electrolyte leakage at optimal and chilling temperatures with SA spraying. The positive correlation of RWC with protein and dry weight shows that plants sprayed with 100 µM Spd and SA and grown under 80 % FC kept their leaf RWC at a high level and thus protein and dry weight were increased at the end of experiment. A similar report by Latif *et al.* (2015) showed that the correlation of RWC was positive and significant with the fresh and dry weight of *Zea mays* L. Farooq *et al.* (2009a) found a positive correlation between RWC and SA treatment.

The correlation between superoxide dismutase and catalase was negative. Superoxide dismutase was significantly affected by the interaction of the treatments but the catalase activity was not affected by the interaction of the treatments. In our experiment, the treatment with SA resulted in the reduction of catalase activity and the promotion of superoxide dismutase, These results agree

with Janda *et al*. (2003) that showed that the application of SA can reduce catalase activity and alteration in this phenomenon leads to the induction of a defense response in plants (Gechev *et al*., 2002). SA was found to enhance the activities of antioxidant enzymes, peroxidase, and superoxide dismutase when sprayed exogenously to the drought-stressed plants of *Lycopersicon esculentum* (Hayat *et al*., 2008). Krantev *et al*. (2008) reported that the exogenous application of SA enhanced the activities of antioxidant enzymes ascorbate peroxidase and SOD with a concomitant decline in the activity of catalase in maize plants.

Table 2. Correlation coefficients between different characteristics in hollyhocks affected by drought stress and foliar spraying.

	1	2	3	4	5	6	7	8
1-SPAD	1							
2-Electrolyte leakage (%)	-0.74 **	1						
3-Relative water content	0.77**	-0.89**	1					
4-Proline	0.19^{ns}	-0.34**	0.48**	1				
5-Total protein	0.75**	-0.75**	0.63**	-0.04^{ns}	1			
6-Superoxide dismutase	0.14^{ns}	-0.36**	0.39**	0.61**	0.06^{ns}	1		
7-Catalase	-0.38**	0.32**	-0.32**	-0.21^{ns}	-0.26*	-0.32**	1	
8-Dry weight	0.75**	-0.86**	0.83**	0.22^{ns}	0.71**	0.39**	-0.38**	1

*, ** and ^{ns}: Significant at $P < 0.05$, $P < 0.01$ and insignificant, respectively.

CONCLUSION

The results showed that physiological and morphological traits of the plants were significantly affected by drought stress. Generally, 100 µM Spd and SA treatment improved various morphological, physiological and biochemical features of hollyhock significantly. From the physiological point of view, 400 µM SA did not have a significant impact on RWC while it significantly decreased electrolyte leakage, proline, and total protein. Applying Spd and SA improved the measured traits under drought conditions. It is concluded that Spd and SA may be used as a potential growth regulator to improve plant drought stress tolerance. However, the amount of exogenous application of Spd and SA has to be refined with further experimentation separately for each crop.

Literature Cited

Adam, S. and Murthy, S. 2013. Role of polyamines and their effect on photosynthesis in plants. Pharmaceutical, Biological and Chemical Sciences, 4: 596-605.

Agarwal, S. and Pandey, V. 2004. Antioxidant enzyme responses to NaCl stress in *Cassia angustifolia*. Plant Biology, 48: 555-560.

Agnes, S., Csiszar, J., Bajkan, S., Gemes, K., Horvath, F., Erdei, L., Deer, A.K., Simon, M.L. and Tari, I. 2005. Role of salicylic acid pre- treatment on the acclimation of tomato plants to salt and osmotic stress. Acta Biologica Szegediensis, 49: 123-125.

Ahmadiyan, A., Ghanbari, A., Siahsar, B., Heydari, M., Ramroudi, M. and Mousavi Nik, S.M. 2010. Effects of residual of chemical fertilizer, cattle manure and compost on yield, yield components, some physiological characteristics and essential oil content of *Matricaria*

chamomilla under drought stress condition. Iranian Journal of Field Crops Research, 8: 668-676.

Ahmed, B., Roy, C.D., Subramaniam, S.A., Ganie, D., Kwatra, D., Dixon, D., Anant, A., Zarqar, M.A. and Umar, S. 2016. An ornamental plant targets epigenetic signaling to block cancer stem cell driven colon carcinogenesis. Carcinogenesis, 37: 385-396.

Almeida, V.O., Batista, K.A., Di-Medeiros, M.C.B., Moraes, M.G. and Fernandes, K.F. 2019. Effect of drought stress on the morphological and physicochemical properties of starches from *Trimezia juncifolia.* Carbohydrate Polymers, 212: 304-311.

Alscher, R., Heath, A., Erturk, N. and Heath, L.S. 2002. Role of superoxide dismutases (SODs) in controlling oxidative stress in plants. Journal of Experimental Botany, 53: 1331-1341.

Bajji, M., Kinet, J.M. and Lutts, S. 2002. Osmotic and ionic effects of NaCl on germination, early seedling growth and ion content of *Atriplex halimus* (Chenopodiaceae). Canadian Journal of Botany, 80: 297-304.

Barranco, D., Ruiz, N. and Gomez del Campo, M. 2005. Frost tolerance of eight olive cultivars. HortScience, 40: 558-560.

Bates, L.S., Waldren, R.P. and Teare, I.D. 1973. Rapid determination of free proline for water stress studies. Plant and Soil, 39: 205-207.

Bradford, M.M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry, 72: 248-54.

Chance, B. and Maehly, A.C. 1955. Assay of catalase and peroxidase. Methods in Enzymology, 2: 764-775.

Cleland, C.F. and Ajami, A. 1974. Identification of the flower-inducing factor isolated from aphid honeydew as being salicylic acid. Plant Physiology, 54: 904-906.

DaCosta, M. and Huang, B. 2007. Changes in antioxidant enzyme activities and lipid peroxidation for bent-grass species in responses to drought stress. Journal of the American Society for Horticultural Science, 132: 319-326.

El Tayeb, M.A. and Ahmed, N.L. 2010. Response of wheat cultivars to drought and salicylic acid. American-Eurasian Journal of Agronomy, 3: 01-07.

Eraslan, F., Inal, A., Gunes, A. and Alpaslan, M. 2007. Impact of salicylic acid on growth, antioxidant activity and physiology of carrot plants subjected to combined salinity and boron toxicity. Scientia Horticulturae, 113: 120-128.

Faize, M., Burgos, L., Faize, L., Piqueras, A., Nicolas, E., Barba-Espin, G., Clemente-Moreno, M.J., Alcobendas, R., Artlip, T. and Hernandez, J.A. 2011. Involvement of cytosolic ascorbate peroxidase and Cu/Zn-superoxide dismutase for improved tolerance against drought stress. Journal of Experimental Botany, 62: 2599-2613.

Fang, Y. and Xiong, L. 2015. General mechanisms of drought response and their application in drought resistance improvement in plants. Cellular and Molecular Life Sciences, 72: 673–689.

Fariduddin, Q., Hayat, S. and Ahmad, A. 2003. Salicylic acid influences net photosynthetic rate, carboxylation efficiency, nitrate reductase activity, and seed yield in *Brassica juncea*. Photosynthetica, 41: 281-284.

Farjadi-Shakib, M., Naderi, R. and Mashhadi Akbar Boojar, M. 2012. Effect of salicylic acid application on morphological, physiological and biochemical characteristics of *Cyclamen persicum* Miller. Annals of Biological Research, 3: 5631-5639.

Farooq, M., Aziz Basra, T. S. M. A., Cheema, M. A. and Rehman, H. 2009a. Chilling tolerance in hybrid maize induced by seed priming with salicylic acid. Journal of Agronomy and Crop Science, 194: 161-168.

Farooq, M., Wahid, A., Kobayashi, N., Fujita D. and Basra, S.M.A. 2009b. Plant drought stress: Effects, mechanisms and management. Agronomy for Sustainable Development, 29: 185-212.

Farooq, M., Wahid, A., Lee, D.J., Cheema, S.A. and Aziz, T. 2010. Comparative time course action of the foliar applied glycinebetaine, salicylic acid, nitrous oxide, brassinosteroids and spermine in improving drought resistance of rice. Agronomy and Crop Science, 196: 336-345.

Gechev, T., Gadjev, I., van-Breusegem, F., Inze, D., Dukiandjiev, S., Toneva, V. and Minkov, I. 2002. Hydrogen peroxide protects tobacco from oxidative stress by inducing a set of antioxidant enzymes. Cellular and Molecular Life Sciences, 59: 708-714.

Ghodke, P.H., Andhale, P.S., Gijare, U.M., Thangasamy, A., Khade, Y.P., Mahajan, V. and Singh, M. 2018. Physiological and biochemical responses in onion crop to drought stress. International Journal of Current Microbiology and Applied Sciences, 7: 2054-2062.

Giannopolitis, C.N. and Reis, S.K. 1977. Superoxide dismutase I. Occurrence in higher plants. Plant Physiology, 59: 309-314.

Gill, S.S. and Tuteja, N. 2010. Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. Plant Physiology and Biochemistry, 48: 909-930.

Guo, Y., Tian, Y., Liu, S.S., Wang, S.S. and Sui, W.Q.N. 2018. Energy dissipation and antioxidant enzyme system protect photosystem II of sweet sorghum under drought stress. Photosynthetica, 56: 861-872.

Hayat, S., Hasan, S.A., Fariduddin, Q. and Ahmad, A. 2008. Growth of tomato (*Lycopersicon esculentum*) in response to salicylic acid under water stress. Journal of Plant Interactions, 3: 297–304.

Hoagland, D.R. and Arnon, D.I. 1950. The water culture method for growing plants without soil. College of Agriculture, University of California in Berkeley, Calif. 32 pages.

Horvath, E., Szalai, G. and Janda, T. 2007. Induction of abiotic stress tolerance by salicylic acid signaling. Journal of Plant Growth Regulation, 26: 290-300.

Hussain, L., Akash, M.S.H., Tahir, M., Rehman, K. and Ahmed, K.Z. 2014. Hepatoprotective effects of methanolic extract of *Alcea rosea* against acetaminophen-induced hepatotoxicity in mice. Bangladesh Journal of Pharmacology, 9: 322-327.

Janda, T., Szalai, G., Rios-Gonzalez, K., Veisz, O. and Paldi, E. 2003. Comparative study of frost tolerance and antioxidant activity in cereals. Plant Science, 164: 301-306.

Jiang, M. and Zhang, J. 2001. Effect of abscisic acid on active oxygen species, antioxidative defence system and oxidative damage in leaves of maize seedlings. Plant Cell Physiology, 42: 1265- 1273.

Kang, D.J., Seo, Y.J., Lee, J.D., Ishii, R., Kim, K.U., Shin, D.H., Park, S.K., Jang, S.W. and Lee, I.J. 2005. Jasmonic acid differentially affects growth, ion uptake and abscisic acid concentration in salt-tolerant and salt-sensitive rice cultivars. Journal of Agronomy and Crop Science, 191: 273-282.

Khalighi, A., Naderi, R., Salami, A.R., Babalar, M., Rohollahi, E. and Khaleghi, Gh.R. 2016. Evaluating the effect of spermidine and salicylic acid on reduce drought stress injuries of one-year-old *Maclura pomifera* seedlings. Journal of Crop Improvement, 18: 231-244.

Khan, W., Prithviraj, B. and Smith, D.L. 2003. Photosynthetic responses of corn and soybean to foliar application of salicylates. Journal of Plant Physiology, 160: 485-492.

Koyro, H., Ahmad, P. and Geissler, N. 2012. Abiotic stress responses in plants: An overview. Environmental Adaptations and Stress Tolerance of Plants in the Era of Climate Change, pp. 1-28.

Krantev, A., Yordanova, R., Janda, T., Szalai, G. and Popova, L. 2008. Treatment with salicylic acid decreases the effect of cadmium on photosynthesis in maize plants. Journal of Plant

Physiology, 165: 920-931.
Krasensky, J. and Jonak, C. 2012. Drought, salt, and temperature stress-induced metabolic rearrangements and regulatory networks. Experimental Botany, 63: 1593-1608.
Larkunthod, P., Nounjan, N., Siangliw, J.L., Toojinda, T., Sanitchon, J., Jongdee, B. and Theerakulpisut, P. 2018. Physiological responses under drought stress of improved drought-tolerant rice lines and their parents. Notulae Botanicae Horti Agrobotanici Cluj-Napoca, 46: 679-687.
Latif, F., Ullah, F., Mehmood, S., Khattak, A., Khan, A.U., Khan, S. and Husain, I. 2015. Effects of salicylic acid on growth and accumulation of phenolics in *Zea mays* L. under drought stress. Acta Agriculturae Scandinavica, Section B - Soil and Plant Science, 66: 335-323.
Lee, B. and Zhu, J.K. 2010. Phenotypic analysis of *Arabidopsis* mutants: Electrolyte leakage after freezing stress. Cold Spring Harbour Protocols, No. 4970.
Martin-Mex, R., Villanueva-Couoh, E., Herrera-Campos, T. and Larque-Saavedra, A. 2005. Positive effect of salicylates on the flowering of African violet. Scientia Horticulturae, 103: 499-502.
Martin-Mex, R., Villanueva-Couoh, E., Uicab-Quijano, V. and Larque-Saavedra, A. 2003. Positive effect of salicylic acid on the flowering of gloxinia. Proceedings 31st Annual Meeting Plant Growth Regulation Society of America, Vancouver, Canada. 3-6 August 2003, p. 149-151.
Merwad, A.M.A., Desoky, E.M. and Rady, M.M. 2018. Response of water deficit-stressed *Vigna unguiculata* performances to silicon, proline or methionine foliar application. Scientia Horticulturae, 228: 132-144.
Michaletii, N., Naghavi, M.R., Toorch, M., Zolla, L. and Rinaldocci, S. 2018. Metabolomics and proteomics reveal drought-stress responses of leaf tissues from spring-wheat. Scientific Report, 8: 5710-5728.
Mustafavi, S.H., Shekari, F., Nasiri, Y. and Hatami-Maleki, H. 2015. Nutritional and biochemical response of water-stressed valerian plants to foliar application of spermidine. Biological Forum – An International Journal, 7: 1811-1815.
Noman, A., Aqeel, M., Deng, J., Khalid, N., Sanaullah, T. and Shuilin, H. 2017. Biotechnological advancements for improving floral attributes in ornamental plants. Frontiers in Plant Science, 8: 1-15.
Oraee, A., Tehraifar, A., Nezami, A. and Shoor, M. 2018. Effects of drought stress on cold hardiness of non-acclimated viola (*Viola× wittrockiana* 'Iona Gold with Blotch') in controlled conditions. Scientia Horticulturae, 238: 98-106.
Pakravan, M. and Ghahreman, A. 2003. Some new combinations and synonyms in *Alcea* (Malvaceae) from Iran. Annalen des Naturhistorischen Museums in Wien, 104: 713-716.
Pan, Q., Zhan, J., Liu, H., Zhang, J., Chen, J., Wen, P. and Huang, W. 2006. Salicylic acid synthesized by benzoic acid 2- hydroxylase participates in the development of thermos-tolerance in pea plants. Plant Science, 171: 226-233.
Reddy, A.R., Chaitanya, K.V. and Vive Kanandan, M. 2004. Drought-induced responses of photosynthesis and antioxidant metabolism in higher plants. Journal of Plant Physiology, 161: 1189-1202.
Rezayian, M., Niknam, V. and Ebrahimzadeh, H. 2018. Improving tolerance against drought in canola by penconazole and calcium. Pesticide Biochemistry and Physiology, 149: 123-136.
Sawhney, V. and Singh, D.P. 2002. Effect of chemical desiccation at the post-anthesis stage on some physiological and biochemical changes in the flag leaf of contrasting wheat genotypes. Field Crops Research, 77: 1-6.
Turner, N.C. 1981. Techniques and experimental approaches for the measurement of plant water status. Plant and Soil, 58 (1): 339-366.

Turner, N.C. 2018. Turgor maintenance by osmotic adjustment – 40 years of progress. Journal of Experimental Botany, 69: 3223-3233.

Tyagia, J., Varma, A. and Pudake, R.N. 2017. Evaluation of comparative effects of arbuscular mycorrhiza (*Rhizophagus intraradices*) and endophyte (*Piriformospora indica*) association with finger millet (*Eleusine coracana*) under drought stress. European Journal of Soil Biology, 81:1-10.

Ullah, F., Bano, A. and Nosheen, A. 2012. Effects of plant growth regulators on growth and oil quality of canolas (*Brassica napus* L.) under drought stress. Journal of Botany, 44: 1873-1880.

Vahdati Mashhadian, N., Tehranifar, A., Bayat, H. and Selahvarzi, Y. 2012. Salicylic and citric acid treatments improve the vase life of cut chrysanthemum flowers. Journal of Agricultural Science and Technology, 14: 879-887.

Zarghami, M., Shoor, M., Ganjali, A., Moshtaghi, N. and Tehranifar, A. 2014. Effect of salicylic acid on morphological and ornamental characteristics of petunia hybrid at drought stress. Indian Journal of Fundamental and Applied Life Sciences, 4: 523-532.

Zhang, T., Hub, Y., Zhang, K., Tianc, C. and Guoa, J. 2018. Arbuscular mycorrhizal fungi improve plant growth of *Ricinus communis* by altering photosynthetic properties and increasing pigments under drought and salt stress. Industrial Crops and Products, 17: 13-19.

Zhang, C.M., Zou, Z.R., Huang, Z. and Zhang, Z.X. 2010. Effects of exogenous spermidine on photosynthesis of tomato seedlings under drought stress. Agricultural Research in the Arid Areas, 3: 182-187.

How to cite this article:
Oraee, T., Shoor, M., Tehranifar, A. and Nemati, H. 2019. Alleviation of drought stress effects by exogenous application of Spermidine and Salicylic Acid on Hollyhock (*Alcea rosea*). *Journal of Ornamental Plants,* 9(4)*,* 303-318.
URL: **http://jornamental.iaurasht.ac.ir/article_667494_80e42ff45dcdd1d7f7c13a72ceac1fe6.pdf**