UNDER DROUGHT STRESS, FOLIAR APPLICATION OF PUTRESCINE ENHANCES PHOTOSYNTHETIC ACTIVITIES, STOMATAL CLOSURE AND DROUGHT TOLERANCE IN ORNAMENTAL SUNFLOWER (*HELIANTHUS ANNUS* L.) PLANT

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Abstract. Plants' resistance to drought stress largely depends on their polyamine contents. The present study aims to investigate the enhancing effects of foliar application of Putrescine (Put) on short-term tolerance against drought stress in ornamental sunflowers (*Helianthus annus* L. cv. Sunbright Kids). Plants were irrigated at 40 and 80% field capacity (FC). Foliar spray of Put (0, 75 and 150 mgl^{-1}) was applied twice during the growing period. Water stress at 40% FC, compared to 80% FC, significantly affected plant physiological characteristics. While the exogenous application of Put, in comparison with the control, significantly increased chlorophyll b, total chlorophyll, carotenoids, soluble carbohydrate, leaf potassium (K) and nitrogen and root K contents, photosynthetic rate, membrane stability index and photosystem II quantum florescent efficiency (F_v/F_m), it significantly decreased stomatal conductance of CO_2 (g_s) and transpiration rate (T_r). The results confirmed that the foliar application of Put protected plants against drought stress by promoting plants RWC, MSI, Chl and Pn. Accordingly, the reduction in stomatal g_s and K^+ content may lead to stomatal closure and reduction in T_r. Stomatal images are in agreement with g_s and T_r reduction. It was concluded that in order to improve the sunflower plants tolerance to drought stress, the foliar application of commercial Put (75 mgl^{-1}) is recommended for this cultivar.
Keywords: *carbohydrate, chlorophyll, polyamine, transpiration rate, water deficiency*

Introduction

Almost one third of the Earth's surface is classified as arid and semi-arid. Such areas are subject to periodic drought during growing season which affects plants growth and development. Plants respond to drought stress by altering their cellular metabolism and invoking their defense mechanism(s). Morphological and physiological changes and also biochemical processes help plants tolerate drought stress (Bohnert and Jensen, 1996; Zhang et al., 2018). The effects of drought stress on plants vary from species to species (Chaitanya et al., 2003; Elansary, 2017; Liang et al., 2020). Physiological processes such as stomatal closure and decrease in photosynthesic rate as well as a change in water transport within the plant will also take place under prolonged drought stress condition (Figueiredo et al., 1999; Li et al., 2017). Stomatal closure will reduce the production of protective detoxification proteins and low production of these proteins will hamper short-term plants dehydration tolerance (Benesova et al., 2012). Reduction in leaf size is common means for plants to adjust to drought conditions and thus to prevent stomatal closure (Rajan et al., 2010). Leaf area reduction is accompanied by an increase in leaf

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vein density and stomatal density which later contributes to a higher stomatal conductance per unit leaf surface area (Brodribb and Jordan, 2011). Another important mechanism in adaptation of plant to drought condition is the accumulation of Abscisic acid (ABA) since it modifies plants endogenous polyamine (PA) content (Liu et al., 2005). Biosynthesis of ABA leads both to stomata closure and the modulation of ABA-responsive gene, which result in drought resistance (Yamaguchi–Shinozaki and Shinozaki, 2006). In plants, reactive oxygen species (ROS) are continuously produced (even under physiological steady state conditions) in cell organelles involved in active electron transport. These ROS formations may be increased under drought stress which can damage proteins, membrane lipids and photosynthetic pigments. These ROS must be efficiently scavenged and maintained at non-damaging levels to allow cell survival (McCord, 2000). Reduced relative water content (RWC) and leaf water potential (Ψ_{leaf}) under drought stress triggers accumulation of different types of compatible solutes (Hameed and Ashraf, 2008). Polyamines (PAs) are multifunctional compounds which have been shown to be involved in a broad range of physiological functions in plant growth and development. Polyamine accumulation takes place when plants are subjected to stress. Under these conditions, modulation of the PA biosynthetic pathway enhances stress tolerance in plants (Kanayama and Kochetov, 2015). Polyamines (PAs) belong to a group of osmotically active substances playing an important role in drought tolerance. They affect osmotic adjustment, the maintenance of membrane stability and free radical scavenging (Bouchereau et al., 1999; Ebeed et al., 2017; Zhao et al., 2021). The cationic nature of PAs, aliphatic actions with low molecular weight that can be found in all living organisms, leads to the stability and permeability of membranes by binding to the negatively charged head groups of phospholipids or other anionic sites on membranes (Kanayama and Kochetov, 2015). Another crucial function of PA is decreasing the oxidative stress by improving the antioxidant molecules and antioxidant enzyme activities, which protect cell membrane, proteins and DNA/RNA from degradation, since all abiotic stresses lead to oxidative stress (Kanayama and Kochetov, 2015). Compared to susceptible plants, stress tolerant plants have more capability of synthesizing PAs in response to stress and can increase their endogenous polyamine level by a factor of two or three in response to stress (Kasukabe et al., 2004). Exogenous application of PAs has induced drought tolerance of cucumber (*Cucumissativus*) (Kubis, 2008), rice (*Oryza sativa*) (Farooq et al., 2009) and Creeping bentgrass (*Agrostis stolonifera*) (Shukla et al., 2015). Among the PAs, Putrescine (Put) accumulation is crucial to improve drought tolerance in Arabidopsis, since the levels of Spermidine (Spd) and Spermine (Spm) are extremely decreased during dehydration. That is the reason the investigators consider Put as a protective component against stress in Arabidopsis (Alcázar et al., 2010). Scientists believe that Put and ABA positively regulate each other's biosynthesis under abiotic conditions (Alcázar et al., 2010). Putrescine can be degraded by diamine oxidase to produce H_2O_2, which elevates the Ca^{2+} level in guard cells and induces stomatal closure in *Vicia faba* (An et al., 2008). It has been reported that under relatively mild osmotic stress the endogenous Put can be increased by about 83% and decreased the maximum photosystem II photochemical efficiency by about 14%. Application of 1 mM Put one hour before the exposure of plant to osmotic stress, protects plants photochemical capacity and inhibits loss of water. In one previous study the key role of Put in the modulation of plant tolerance against osmotic stress has confirmed (Kotakis et al., 2014). Improving drought tolerance traits in plants is of great importance in ornamental plants in arid and semi-arid landscape of the world. Based on the above statement and

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considering Iran climatic conditions and the exposure of plants in landscape to drought stress during growing season, the aim of this investigation was to study the effects of exogenous Put application on ornamental sunflower in inducing its tolerance against drought stress.

Materials and Methods

The experiments were carried out in the greenhouse of the Department of Horticultural Sciences and Landscape, Faculty of Agriculture, Ferdowsi University of Mashhad, Iran, under controlled conditions with relative humidity of 60% at 25°C/18°C (day/night). The light intensity within the greenhouse was set at 500-700 μmol m^{-2} s^{-1}. Plants used in this study were the F_1 seeds of ornamental sunflower (*Helianthus annus* L. cv. Sunbright Kids) purchased from Sakata Company, Japan. The seeds were planted in trays containing coco peat, peat moss, and perlite (1:1:1, v/v/v). When the seedlings were at 6 leaf stage (after one month), they were transplanted into plastic pots containing 4.6 kg of soil, leaf mold, vermicompost and sand (7:1:1:1, v/v/v/v) (*Fig. 1*). Prior to the experiments, the physical properties of the soil mixture used were determined (*Table 1*). Forty days after seeds planting, Putrescine (Put) (0, 75 and 150 mgl^{-1}) was sprayed on the seedlings. The amount sprayed was enough to soak the leaves and the solution started to drip and applied 10 times for each plant. Control plants were sprayed with the same amount of distilled water. As the seedlings entered the reproductive phase (60 days after seed planting), soil moisture contents were maintained at 40% and 80% field capacity (FC). One month after the initial foliar Put spray (70 days after seed planting and 10 days after plants entered the reproductive phase), the second spray was applied, same way as the first one (*Fig. 2*). Recently fully expanded leaves were used for all measurements.

Figure 1. *Ornamental sunflower seedlings used in the experiments*

Table 1. *Some of the physical and chemical characteristics of potting soil*

EC (dS/m)	pH	N (%)	K (mg/Kg)	P (mg/Kg)	Sand (%)	Clay (%)	Silt (%)	FC (%)
3.53	7.15	0.1428	482.3	36.8	42	18	40	31.2

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Figure 2. *Ornamental sunflower plants used in this experiment*

At the end of the experiment, the following physiological parameters were determined:

Photosynthetic rate (Pn), transpiration rate (T_r) and stomatal conductance of CO_2 (g_s)

Pn, T_r and g_s were determined by means of a Portable Photosynthesis System LCi Console (ADC Bioscientific Ltd Co., Hoddesdon, England) with a chamber size of 6.25 cm^2. Measurements were made between 10 AM and 12 noon by clamping the leaves in the leaf chamber, exposed to direct sunlight (*Fig. 3*).

Figure 3. *Portable Photosynthesis System LCi Console used in the experiment*

Photosystem II photochemical efficiency

Photosystem Π quantum florescent efficiency (Fv/Fm) was measured by OS1-FL Modulated Fluorometer (OPTI-SCIENCES Co., USA) (*Fig. 4*).

Stomata size

Recently mature leaves samples were collected in the midday and immediately dried by a freeze dryer (Model BETA 2-8 LD plus, CHRIST Co., Germany) to be examined by

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scanning electron microscope. The freeze dried samples were mounted on normal SEM stubs with a double-sided adhesive tape, coated with 25-nm gold palladium using a Hummer II Sputter Coater (Model SC7620, England) for 150 seconds. The samples were examined by a scanning electron microscope (SEM) (Model LEO 1450VP, Germany) operated with 2.5nm resolution at 20kV. The SEM images of abaxial surfaces were magnified to a fixed resolution (3000× for stomata size). With respect to the images scale bar, measurements were made by means of SEM device.

Figure 4. *OS1-FL Modulated Fluorometer used in the experiment*

Membrane stability index (MSI)

Leaf cells membranes damage was determined by recording their electrolyte leakage (EL) as described by Valentovic et al. (2006) with some modifications. Plant materials (0.5 g) were washed with deionized water and placed in tubes containing 20 ml deionized water and incubated for 24 h at 25°C. Subsequently, the electrical conductivity of the leakage solution (L_1) was measured. Samples were then autoclaved at 120°C for 20 min and the final solution conductivity (L_2) was determined after equilibration at 25°C. Membrane stability index percentage (%MSI) was defined according to Singh et al. (2008) (*Eq. 1*).

$$MSI\ (\%) = [1 - (L_1/L_2)] \times 100 \qquad \text{(Eq.1)}$$

Relative water content (RWC)

To evaluate leaves relative water content, firstly the fresh weight of the samples (10 discs of 0.5 cm diameter each) were measured, and then the samples soaking in 20 ml distilled water at 4˚C in the dark for 24 h. The turgid leaves were quickly and carefully blotted dry and their turgid weight were determined. The weight of the dried leaves were also specified after being oven-dried at 75°C for 24 h. RWC was calculated according to Smart & Bingham (1974) (*Eq. 2*).

$$RWC = [\text{fresh weight- dry weight/ turgid weight – dry weight}] \times 100 \qquad \text{(Eq.2)}$$

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Chlorophylls and carotenoids

Using mortar and pestle, 0.2 gram fresh leaf was ground to a fine pulp in 25 ml 80% acetone. The mixture was centrifuged at 5000 rpm for 10 min (Model Z200A, Hermle Co., Germany) and then the supernatant was transferred to a falcon tube. The precipitate was colorless. The supernatant absorbance was read at 480, 510, 645 and 663 nm against the solvent (80% acetone) as blank by spectrophotometer (Model 6305, JENWAY Co., UK). The amounts of chlorophylls (Arnon, 1949) and carotenoids (Ranganna, 1977) were calculated using the *equations 3, 4, 5 and 6*.

$$\text{mg Chlorophyll a/g tissue} = [12.7\ (A663)-2.69(A645)]\times\frac{V}{1000\times W} \quad \text{(Eq.3)}$$

$$\text{mg Chlorophyll b/g tissue} = [22.9\ (A645)-4.68(A663)]\times\frac{V}{1000\times W} \quad \text{(Eq.4)}$$

$$\text{mg total Chlorophyll/g tissue} = [20.2\ (A645)+8.02\ (A663)]\times\frac{V}{1000\times W} \quad \text{(Eq.5)}$$

$$\text{mg carotenoids/g tissue} = [7.6\ (A480)-1.49(A510)]\times\frac{V}{1000\times W} \quad \text{(Eq.6)}$$

where,
A = Absorbance at specific wave length.
V = Final volume of chlorophyll & Carotenoid in 80% of acetone.
W = Fresh weight of the tissue extracted.

Leaf soluble carbohydrates assay

Fresh leaf samples were freeze dried by a freeze dryer (Model BETA 2-8 LD plus, CHRIST Co., Germany) and then ground (*Fig. 5*). A few drops of ethanol (80%) were added to 0.1 g dry leaf powder. Subsequently, 25 ml aqueous ethanol (5 ml water + 20 ml 80% ethanol) was added and mixed vigorously by shaking. After centrifuging at 5000 rpm for 15 min (Model Z200A, Hermle Co., Germany), the supernatant was separated, decolorized by 0.01 g activated carbon, and filtrated. Filtrate volume was made to 100 ml with water (McCready et al., 1950). About 1 ml of supernatant was placed in falcon tube and 10 ml of anthrone solution (0.15%) were added. Finally, the samples were heated at 95°C for 8 minutes. The tubes were immediately transferred into an ice bath and cooled down to room temperature. The absorbance of the sample solutions was recorded at 625 nm by a spectrophotometer (Model CE2502, CECIL Co., UK). The total sugar concentrations of the samples were calculated using the calibration curve drawn for glucose standard solutions (Ebell, 1970).

Proline

Analytical proline was used as standard. Acid ninhydrin was prepared by warming 1.25 g ninhydrin in 30 ml glacial acetic acid plus 20 ml 6 M phosphoric acid, agitated until dissolved. Fresh leaf tissue (0.1 g) was homogenized in 10 ml 3% aqueous sulfosalicylic acid followed by the filtration of the homogenate through Whatman filter paper. 2 ml of filtrate reacted with 2 ml of ninhydrin acid and 2 ml of glacial acetic acid was added in a test tube for 1 h at 100°C.The reaction terminated in an ice bath. The reaction mixture was extracted with 2 ml toluene, mixed vigorously with a test tube stirrer

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for 15-20 seconds. The chromophore containing toluene was aspirated from the aqueous phase, warmed to room temperature. The absorbance was read at 520 nm using toluene as blank. Using standard curve, proline concentration was calculated on a fresh weight based on Bates (1973) (*Eq. 7*).

$$[(\text{gproline/ml} \times \text{ml toluene}) / 115.5\ \mu\text{g/}\mu\text{mole}] / [(\text{g sample}) / 5] = \mu\text{molesproline/g of fresh weight material} \quad \text{(Eq.7)}$$

Figure 5. *Freeze dryer used in the experiment*

Nitrogen (N) content

Plant tissues N content was determined by Kjeldahl method (Horneck and Miller, 1998). In this method, N in plant tissues is converted into NH_4 by wet oxidation of organic matter using H_2S0_4 and digestion catalyst. Ammonium was specified through distilling it into boric acid and titration.

Potassium (K) content

A sample of plant material (1 g) was dried-ashed in Muffle furnace at 500°C, then extracted by 1N HCl (Miller, 1998). Next, K content was determined by flame photometer (Model PFP7, Jenway Co., UK).

DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging activity (leaf non-enzymatic antioxidants)

Using mortar and pestle, 0.2 gram fresh leaf was homogenized at 4°C in 2.0 ml of absolute ethanol (i.e. ethanol solution). A 0.5 ml aliquot was mixed with a 0.5 mM DPPH ethanol solution (0.25 ml) and 100 mM acetate buffer (pH 5.5; 0.5 ml). After standing for 30 min, the absorbance of the mixture was measured at 517 nm (Abe et al., 1998).

Statistical analysis

The experiments were conducted as a factorial (2×3) in complete randomized block design with four replications and 8 plants in each replicate. Statistical differences between measurements were analyzed following the analysis of variance (ANOVA) using Minitab 16.0 software. LSD test was used to compare the means and differences ($P \leq 0.05$).

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Results

The results of drought stress simple effects on sunflower plants physiological parameters are shown in *Tables 2, 3 and 4 and Figure 6*. drought stress at 40% FC in comparison with 80% FC reduced significantly chlorophyll a content (Chl a) (25%), chlorophyll b content (Chl b) (50%), total chlorophyll content (total Chl) (45.45%), carotenoids content (33.33%), soluble carbohydrate content (32.2%) (*Table 2*), leaf K content (25.81%), leaf N content (15.38%) (*Table 3*), photosynthetic rate (Pn) (23.38%) (*Figure 6*), stomatal conductance of CO_2 (g_s) (66.67%), membrane stability index (MSI) (9.19%), transpiration rate (T_r) (23.40%), leaf relative water content (RWC) (3.53%) and photosystem II quantum florescent efficiency (F_v/F_m) (6.20%) (*Table 4*). However, it significantly increased both leaves proline content (42.66%) and non-enzymatic antioxidants (61.62%) (*Table 2*). The decrease in stomatal pore sizes is shown in *Figures 7 and 8*.

Table 2. *Effects of drought stress on some physiological parameters*

Drought stress (FC%)	Characteristics						
	Chlorophyll a (mg/gfw)	Chlorophyll b (mg/gfw)	Total Chlorophyll (mg/gfw)	Carotenoid (mg/gfw)	Soluble carbohydrate (mg/gdw)	Proline (µg/gfw)	Leaf non-enzymatic antioxidants (%)
40	0.4 b∗	0.2 b	0.6 b	0.2 b	21.9 b	147.8 a	48.0 a
80	0.8 a	0.4 a	1.1 a	0.3 a	32.3 a	103.6 b	29.7 b

∗: Means with different letters in a column are statistically significant based on LSD test at P ≤ 5% level

Table 3. *Effects of drought stress on some physiological parameters*

Drought stress (FC%)	Characteristics			
	Leaf N content (%)	Root N content (%)	Leaf K content (%)	Root K content (%)
40	1.1 b∗	2.2 a	2.3b	1.2a
80	1.3 a	2.2 a	3.1 a	1.3 a

∗: Means with different letters in a column are statistically significant based on LSD test at P ≤ 5% level

Table 4. *Effects of drought stress on some physiological parameters*

Drought stress (FC%)	Characteristics				
	Membrane stability Index (%)	Transpiration rate (mmolH2O $m^{-2}s^{-1}$)	Leaf relative water content (%)	Photosystem Π quantum florescent efficiency	stomatal conductance of CO_2 (molCO_2 $m^{-2}s^{-1}$)
40	23.70 b∗	7.20b	57.40 b	0.67 b	0.50 b
80	26.10 a	9.40a	59.50 a	0.71 a	1.50 a

∗: Means with different letters in a column are statistically significant based on LSD test at P ≤ 5% level

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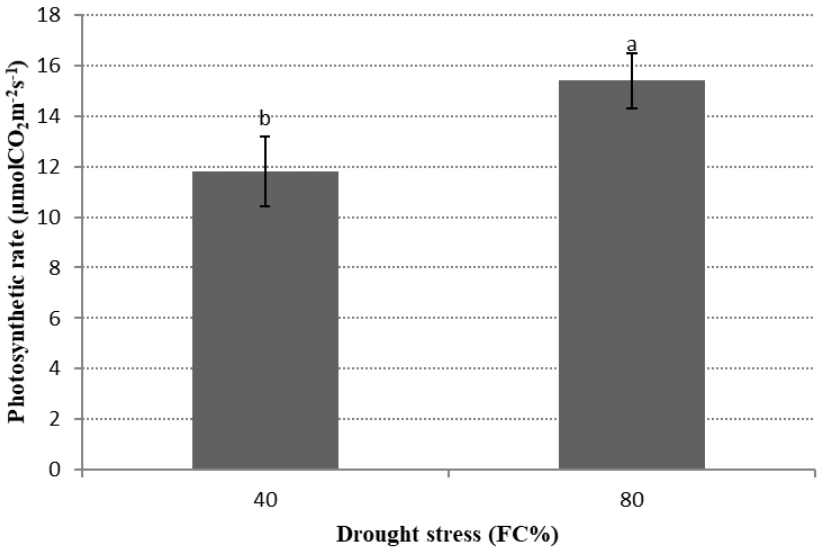

Figure 6. *Effect of drought stress on photosynthetic rate. Data are means ± SE (Standard Errors). Columns with different letters are statistically significant based on LSD test at P ≤ 5% level*

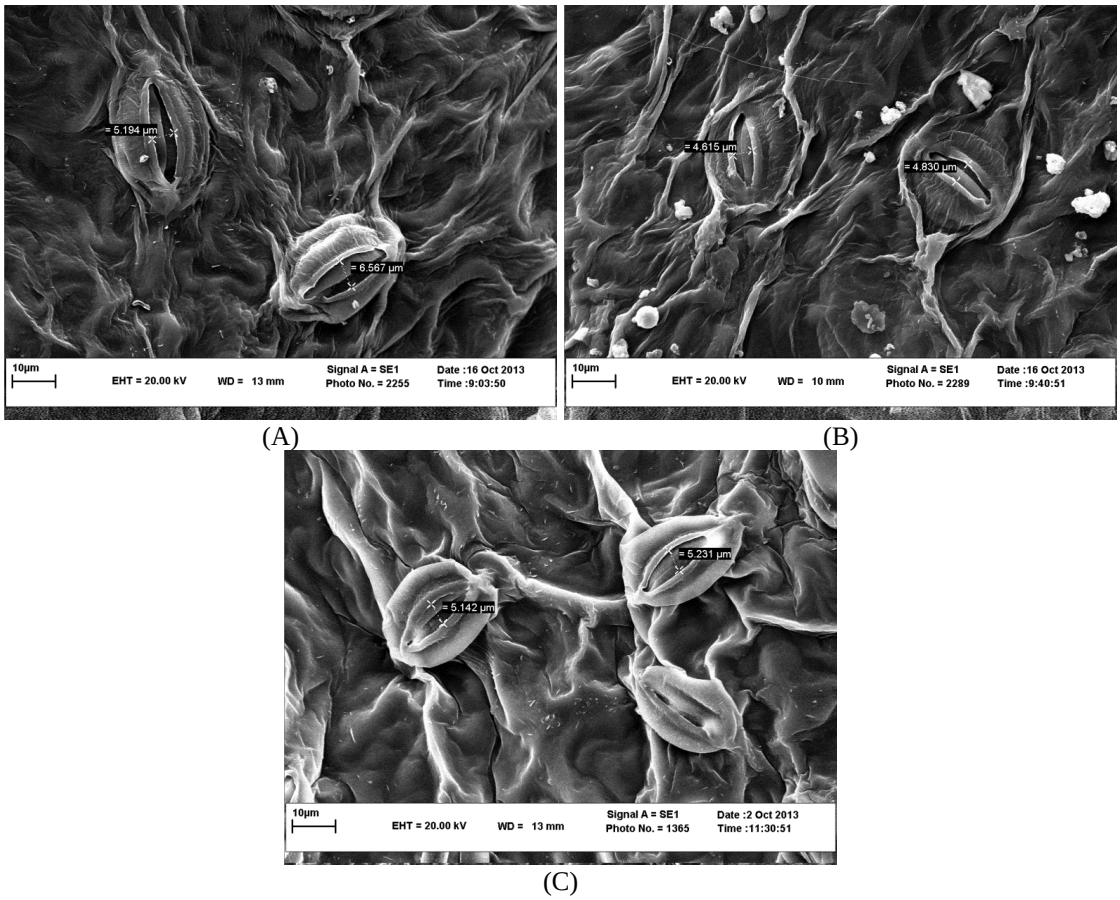

Figure 7. *Stomata SEM images at 3000× magnification. Plants under 80% FC drought stress. (A) 0 (mgl-1) Putrescine, (B) 75 (mgl-1) Putrescine, (C) 150 (mgl-1) Putrescine. The scale bar of the images, were prepared by means of SEM device*

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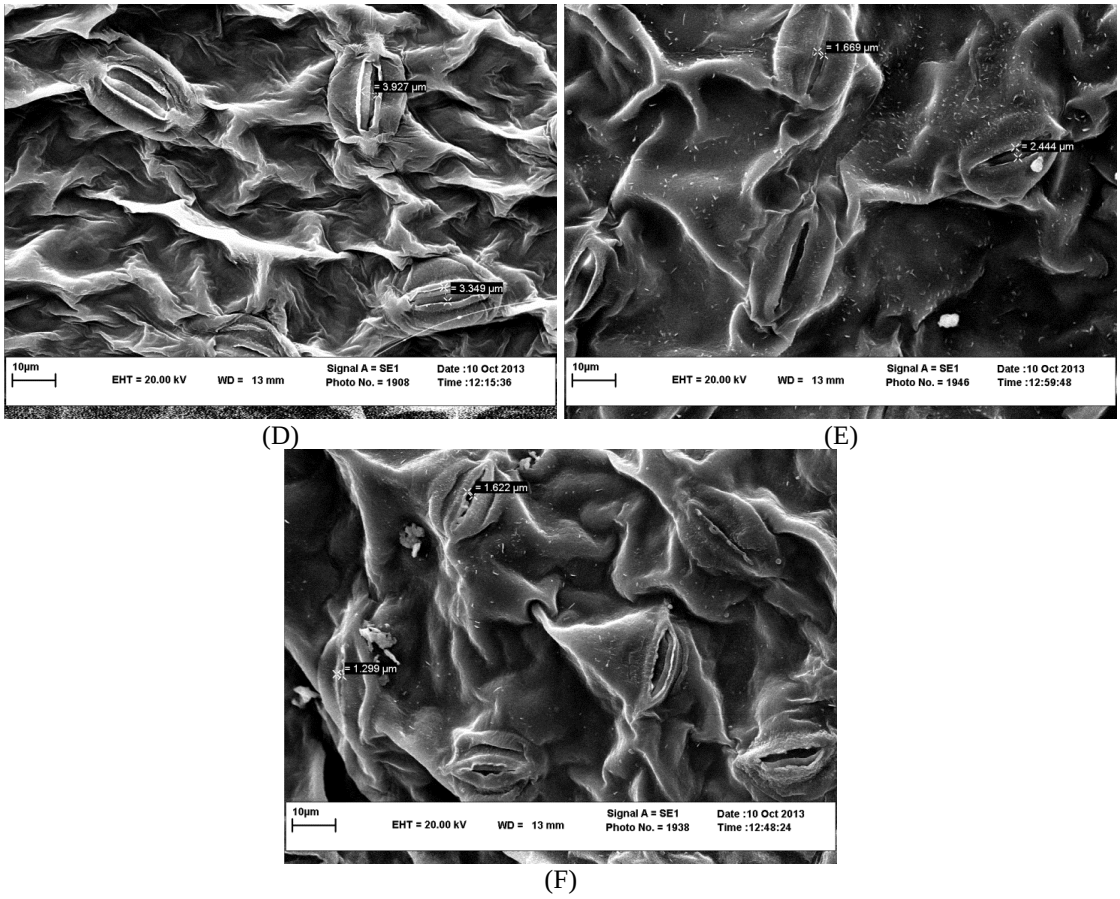

Figure 8. *Stomata SEM images at 3000× magnification. Plants under 40% FC drought stress. (D) 0 (mgl-1) Putrescine, (E) 75 (mgl-1) Putrescine, (F) 150 (mgl-1) Putrescine. The scale bar of the images measurements was prepared by means of SEM device*

The effects of exogenous application of Putrescine (Put) on some physiological parameters of sunflower plants under drought stress are shown in *Tables 5, 6 and 7 and Figure 9*. Put at 75 and 150 mgl^{-1}, as compared with control, significantly increased leaves Chl b (50% and 50%, respectively), total Chl (14.29% and 42.86%, respectively), carotenoids (29.41% and 41.18%, respectively), soluble carbohydrate (35.59% and 9.32%, respectively) (*Table 5*), leaf K content (30.43% and 21.74%, respectively), leaf N content (27.27% and 9.09%, respectively), root K content (66.67% and 55.56%, respectively) (*Table 6*), Pn (34.86% and 40.37%, respectively) (*Figure 9*), MSI (12.02% and 7.72%, respectively) and F_v/F_m (7.62% and 6.86%, respectively). Putrescine application also significantly decreased g_s (16.67% and 25% respectively) and T_r (6.98% and 3.49%, respectively) (*Table 7*). At 75 mgl^{-1}, Put significantly increased leaf RWC (5.23%).The decrease in stomatal pore sizes is shown in *Figures 7 and 8*.

The findings of this study revealed that Put at 75 mgl^{-1}as compared with 150 mgl^{-1} is more effective in increasing carbohydrate, leaves and roots K contents, leaves N content, MSI, RWC and F_v/F_m and in decreasing T_r (*Tables 5, 6 and 7*). However, there were no significant differences between the two levels of Put used on all sunflower plants physiological parameters studied under drought stress conditions (*Tables 5, 6 and 7*).

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Table 5. *Effects of Putrescine on some physiological parameters*

Put(mgl^{-1})	Characteristics						
	Chlorophyll a (mg/gfw)	Chlorophyll b (mg/gfw)	Total Chlorophyll (mg/gfw)	Carotenoid (mg/gfw)	Soluble carbohydrate (mg/gdw)	Proline (µg/gfw)	Leaf non enzymatic Antioxidants (%)
0	0.45 b∗	0.23 b	0.7 b	0.17 b	23.6 b	105.9 a	35.9 a
75	0.66 ab	0.30 ab	0.8 ab	0.22 ab	32.0 a	119.7 a	43.2 a
150	0.69 a	0.32 a	1.0 a	0.24 a	25.8 ab	151.5 a	37.6 a

∗: Means with different letters in a column are statistically significant based on LSD test at P ≤ 5% level

Table 6. *Effects of Putrescine on some physiological parameters*

Put(mgl^{-1})	Characteristics			
	Leaf N content (%)	Root N content (%)	Leaf K content (%)	Root K content (%)
0	1.1b∗	2.2 a	2.3b	0.9 b
75	1.4 a	2.2 a	3.0 a	1.5 a
150	1.2ab	2.2 a	2.8 ab	1.4 ab

∗: Means with different letters in each column are statistically significant based on LSD test at P ≤ 5% level

Table 7. *Effects of Putrescine on some physiological parameters*

Put (mgl^{-1})	Characteristics				
	Membrane stability Index (%)	Transpiration rate ($mmolH_2O\ m^{-2}s^{-1}$)	Leaf relative water content (%)	Photosystem Π quantum florescent efficiency	Stomatal conductance of CO_2 ($molCO_2\ m^{-2}s^{-1}$)
0	23.30 b∗	8.60 a	57.40b	0.66 b	1.20 a
75	26.10 a	8.00 b	60.40 a	0.71 a	1.00ab
150	25.10ab	8.30ab	57.50b	0.70 a	0.90 b

∗: Means with different letters in a column are statistically significant based on LSD test at P ≤ 5% level

The interactions of drought stress and Put application are demonstrated in *Table 8*. The interactions of drought stress levels (40% and 80% FC) and Put concentrations (75 and 150 mgl^{-1}) significantly reduced T_r and g_s. However, there was an increase in MSI (*Table 8* and *Figures 10 and 11*).

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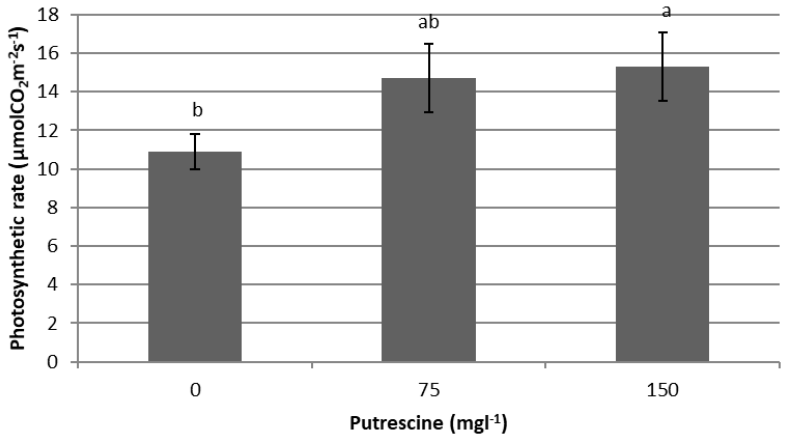

Figure 9. *Effect of Putrescineon photosynthetic rate. Data are means ± SE (Standard Errors). Columns with different letters are statistically significant based on LSD test at P ≤ 5% level*

Table 8. *Interaction effects of different levels of Putrescine and drought stress on some physiological parameters*

Treatments		Characteristics		
		Membrane stability Index (%)	**Transpiration rate ($mmolH_2O\ m^{-2}s^{-1}$)**	**stomatal conductance of CO_2 ($molCO_2\ m^{-2}s^{-1}$)**
Drought stress	Put*			
DS1	0	$21.96\pm0.85^{b**}$	8.43 ± 1.15^{b}	0.60 ± 0.15^{bc}
	75	23.55 ± 2.87^{b}	6.48 ± 0.42^{c}	0.30 ± 0.12^{c}
	150	25.48 ± 3.31^{ab}	6.82 ± 0.64^{c}	0.60 ± 0.28^{bc}
DS2	0	24.71 ± 0.95^{ab}	8.75 ± 0.69^{ab}	1.70 ± 0.33^{a}
	75	28.75 ± 1.47^{a}	9.61 ± 1.07^{a}	1.70 ± 0.06^{a}
	150	24.80 ± 3.64^{ab}	9.69 ± 1.06^{a}	1.10 ± 0.01^{b}

∗ Put (0, 75, 150 mgl^{-1}), DS (Drought stress): DS1: 40%FC; DS2: 80%FC, ∗∗ Data are means ± SD (standard deviation). Means with different letters in a column are statistically significant based on LSD test at P ≤ 5% level

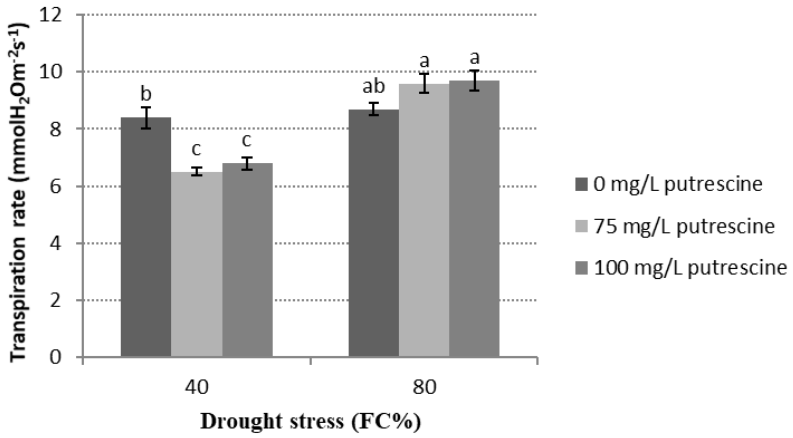

Figure 10. *Interaction effect of drought stress and Putrescine levels on transpiration rate. Data are means ± SE (Standard Errors). Columns with different letters are statistically significant based on LSD test at P ≤ 5% level*

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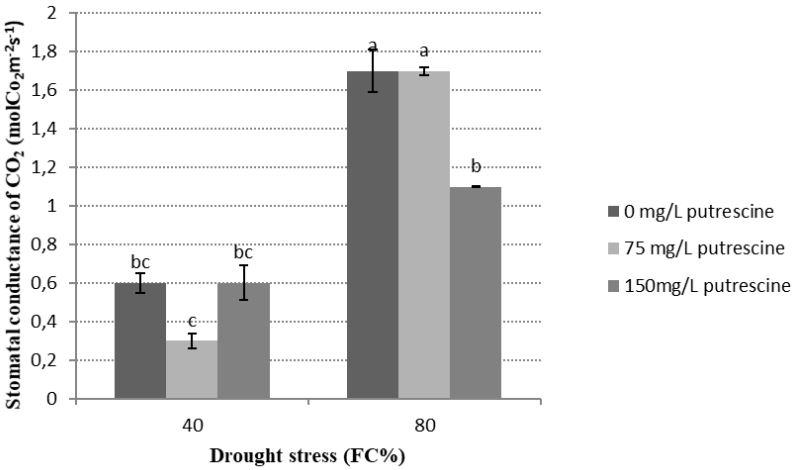

Figure 11. *Interaction effect of drought stress and Putrescine levels on stomatal conductance of CO2. Data are means ± SE (Standard Errors). Columns with different letters are statistically significant based on LSD test at P ≤ 5% level*

Discussion

Drought stress at 40% FC which reduced RWC (*Table 4*) has caused a wide range of changes including chlorophyll loss and reduction in the rates of photosynthesis. The direct relationship between leaves RWC and the rate of photosynthesis has been reported by other workers (Verslues et al., 2006). More specifically, water deficit suppresses photosynthesis through changes in the photosynthetic electron transport (Goltsev et al., 2012). High amounts of carotenoids have preserved chloroplasts structural integrity and high chlorophyll concentration in the fig under drought stress conditions (Rahemi et al., 2017). Maintaining net photosynthesis (Pn) at higher rates under drought stress can be related to higher chloroplast capacity to fix CO_2 and less structural damages under water deficit conditions (Herppich and Peckmann, 1997). In the current study, drought stress significantly reduced total Chl and Pn (*Table 2 and Figure 6*). The reduction in Chl concentration under drought stress is a typical sign of oxidative stress (Egert and Tevini, 2002). In our study, lower membrane stability under drought stress conditions (40% FC) indicates the extent of damages done to the cell membranes and other organless structures such as chlorophyll molecules in sunflower seedlings. Damages to chloroplast by ROS and/or photodynamic degradation may have caused chlorophylls losses under drought stress as has also been reported by others (Anjum et al., 2011). In our study, the accumulation of proline and the increase in non-enzymatic antioxidants in the leaves failed to prevent the degradation of chlorophylls in the leaves of ornamental sunflower plant under water stress (40% FC). The results of this study are in agreement with the ones reported previously in which proline by scavenging ROS and also as a cell membrane stabilizer has probably protected the cells against oxidative stress during dehydration (Verslues et al., 2006). The reduction in g_s and K^+ content in stomata guard cells will reduce stomata aperture followed by the reduction in transpiration rate. In our study, leaves K^+ concentration significantly decreased under drought stress (*Table 3*). A decrease in guard cells potassium content will cause stomata closure and reduce g_s values leading to low transpiration rates under drought stress. Our results are in agreement with those reported by Kirnak et al. (2001) who demonstrated that leaves K^+ concentration

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decreases by drought stress. PAs have an important role in improving plants tolerance to abiotic stresses. The application of Put significantly increased leaves' total chlorophyll and carotenoid contents in ornamental sunflower plants under drought stress (40% FC) (*Table 2*). Our results are in agreement with those reported by Zeid and Shedeed's (2006) who reported on the effects of Put treatment on total chlorophyll, carotenoids and photosynthesis activity of alfalfa under soil drought stress condition. The studies show that Put, by improving leaves RWC has a protective role in plants against drought stress. It has been reported that higher RWC will lead to an improvement in plants maximum photochemical efficiency index (F_v/F_m) and represents the physiological status of the photosynthetic apparatus (Kotakis et al., 2014). Li et al. (2016) observed that exogenous Spd increased the Chl content of plants under drought stress. Our results show that the application of Put can significantly increase RWC, total Chl content, F_v/F_m and subsequently Pn of ornamental sunflower plants under drought stress (*Tables 5 and 7 and Figure 9*). We also found that Put treatment significantly increased MSI and reduced g_s and T_r. Previous reports have revealed that the drought-induced PAs accumulation in plant cells induces stomatal closure, which results in reduced transpiration and water loss by plants (Liu et al., 2000). PAs act as the regulators of inward K^+ channels in the plasma membrane of *Vicia faba* guard cells and modulate stomatal movement. In *Vicia faba,* Put inhibited stomatal opening (Liu et al., 2000). The prevention of inward K^+ channels by Polyamines in guard cells membrane is concentration-dependent and correlates with stomatal closure. Besides preventing inward-rectifying K^+ channels, Polyamines also inhibits outward-rectifying K^+ channels in root cells (Liu et al., 2014; Baronas and Kurata, 2014). In epidermal, cortical, and xylem parenchyma cells of barley roots, the exogenous application of 1 mM Spd has caused approximately a twofold decrease in inward K^+ current (Zhao et al., 2007). Putrescine acts both as a buffer and as an osmolite, and induces plant cells proline content resulting in the preservation of leaf water content under drought stress conditions; thus, Put either directly or indirectly increases plant cells osmotic pressure (Kotakis et al., 2014). Exogenous application of Putrescine in the present study increased a range of osmotically active molecules/ions such as soluble carbohydrate, proline, potassium and nitrogen within the cell of ornamental sunflower plants (*Tables 5 and 6*). Our results are in agreement with those reported by Delauney and Verma (1993). They found that osmotic adjustment in response to water deficiency is basically achieved by the accumulation of some osmotically active molecules or ions like soluble sugars, sugar alcohols, proline, organic acids, calcium, potassium and chloride ions in the cells. Farooq et al. (2009) showed that exogenous application of PA to rice seedlings improved leaves water status, photosynthesis (Pn) and membrane properties, thus enhancing seedlings drought tolerance. Similar results were obtained in the present study when Put was applied prior to applying drought stress treatments (*Table 7 and Figure 9*). The application of Put improved MSI and Pn rate in the plants under drought stress conditions. In our study, the pre-treatment of ornamental sunflower plants with exogenous Put (75 and 150 mgl^{-1}) significantly increased photosystem II quantum florescent efficiency (*Table 7*). Similar results have been reported by Kotakis et al. (2014): thetreatment of leaf discs with exogenous Put (1 mM) 1 hour before exposing them to PEG osmotic stress protected the maximum PSII photochemical efficiency (Fv/Fm) and inhibited the loss of RWC leaf disks. The interaction of drought stress and Put significantly reduced the transpiration rate and g_s, and increased MSI. Lee (1997) revealed that stress tolerant plants endogenously have more PAs such as Put than drought sensitive ones. It seems that ornamental sunflower plant with low concentration of Put is unable to cope with drought

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stress. Low concentration of Put might have been due to inactivation of enzymes involved in Put biosynthesis pathway by the heavy loss of water under drought stress (Rahemi et al., 2017). However, our data revealed that the amount of Put in the ornamental sunflower plants was too low to act as osmoprotectant, and to be effective, Put must be applied to the plants before exposing them to drought stress.

Conclusion

On the basis of the results obtained in this study, ornamental sunflower (Sunbright Kids cultivar) can be classified as a drought sensitive plant. Exogenous application of Put before drought stress, increases the intercellular titer of Put; protects the photochemical capacity; inhibits water loss, and reduces drought stress injury. Putrescine application under drought stress also induces stomatal closure, which results in reduced transpiration and water loss by plants. The results of this study revealed that RWC, MSI, g_s and T_r are important indices to determine drought tolerance in ornamental sunflower cultivars. It was concluded that ornamental sunflower plants do not probably have the capacity of synthesizing sufficient Put in response to drought stress, and should be treated with Put at 75 mgl^{-1} before being exposed to drought conditions. The authors suggest that the effects of some other types of polyamines on morphological and physiological characteristics of ornamental sunflower under drought stress evaluate in future studies as well as internal polyamines, plant hormones and their relationship with applied putrescine. Furthermore, measurement of the soluble carbohydrates in other plant tissue such as flower, stem and root is recommended. Also measuring the stomatal density in the leave surface and statistical analysis of stomatal density and diameter in the leaves of ornamental sunflower under drought stress are suggested in future studies.

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