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Research Paper

Evaluation of Physiological Responses of Tuberose (*Polianthes tuberosa* L.) to Water Deficit Stress

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Abstract

Water stress is one of the main factors of severe damage and limitation of plant production in Iran. The paper reports a pot experiment conducted by using a factorial experiment based on a completely randomized design with three replications (each replication composed of four pots) to compare the physiological and biochemical characteristics of two varieties of tuberose (double- and single-flower) for their tolerance to water stress. Three water stress levels, including no stress (W_1 = 90% of FC), moderate stress (W_2 = 75% of FC) and severe stress (W_3 = 60% of FC), were applied throughout the experiment. Physiological traits including relative water content (RWC) and electrolyte leakage (EL) and biochemical traits including total chlorophyll (Chl), carotenoid (Car) contents, proline, malondialdehyde (MDA), antioxidant enzymes activity (SOD, APX, and CAT), photosynthesis rate (A), transpiration rate (E), and stomatal resistance (r_s) were investigated. Results showed that the physiological and biochemical characteristics of the tuberose plants were highly affected by the application of water stress. MDA and EL showed a significant correlation with most physiological characteristics. Water stress caused significant increases in RWC, A, E, r_s, Chl, APX, and SOD activities of the leaves of the single-flower cultivar. CAT, MDA, and proline content in the leaves of the double-flower cultivar were significantly higher than those in the leaves of the single-flower cultivar, regardless of water treatments. The results showed that the single-flower cultivar grew better than the double-flower one under stress conditions.

Keywords: Antioxidant enzymes, Electrolyte leakage, Gas exchange, Proline, Relative water content.

INTRODUCTION

Iran is located in the dry and semi-arid zone of the world as signified by its low rate of precipitation and inappropriate spatial and temporal distribution of rainfall. Its average annual rainfall is about 240 mm, which is one-third of the global annual precipitation (Modarres *et al*., 2007). Demand for water consumption is increasing due to population growth (the population of Iran is about 80 million), climate change, excessive utilization of water resources for agriculture, and salinity and pollution of existing water resources (Keshavarz *et al*., 2013).

Growth, productivity, and metabolism of plants are affected by biotic stresses (insects, bacteria, fungi, and viruses) and abiotic stresses (drought, salinity, low and high temperatures, flooding, contaminants, and radiation) (Rahman *et al*., 2004). Water stress is one of the main factors of severe damage to most plants so that it is an important abiotic stress and inhibitor of plant production in many arid and semi-arid regions of the world (Reddy *et al*., 2004; Riaz *et al*., 2013). Water stress affects many physiological processes (growth, photosynthesis, respiration, etc.) (Niu *et al*., 2006).

The onset of water stress is associated with a reduction in osmotic potential at the cellular level. Many plants respond to water stress by accumulating organic compounds such as proline. Severe water deficiency reduces photosynthesis and growth rate. Many biochemical and physiological processes in plants are affected by water stress conditions. The production of reactive oxygen species (ROS) induces lipid peroxidation, protein degradation, and damage to nucleic acids (Anjum *et al*., 2011b; Jackson *et al*., 2009).

Plant cells have a range of protective mechanisms to counteract the destructive effects of various ROS, which enables them to protect cellular structures against the damages of ROS. Cell defense systems include non-enzymatic antioxidants (ascorbate, glutathione, tocopherol, and carotenoids) and enzymatic antioxidants (superoxide dismutase, catalase, peroxidase, and ascorbate peroxidase) (Tanou *et al*., 2009). Identification of biochemical and molecular responses to drought stress is essential for a comprehensive understanding of the resistance mechanisms of plants to water stress conditions (Farooq *et al*., 2009).

In the current situation, there is an urgent need to develop special strategies for ornamental plants. Due to water shortage and increasing use of water in agriculture, the horticultural trend is changing around the world; many water-saving techniques are suitable like xeriscaping, the use of drought-tolerant plant species, the application of plant growth regulators, the application of pressurized irrigation systems, and the limited irrigation method. A sustainable approach to coping with drought situation is the introduction and development of drought-tolerant ornamental plant species. The water requirement of resistant plant species is about half the water requirement of susceptible plants *(*Riaz *et al.*, 2013).

Tuberose (*Polianthes tuberosa* L.) from the family Agavaceae is one of the aromatic bulbous flowers in tropical and subtropical areas (Barba-Gonzalez *et al*., 2012). Tuberose is commercially grown for its fragrant cut flowers in Iran (Shoor *et al*., 2005). Tuberose is native to Mexico from where it was transferred to Europe and other parts of the east. In Iran, 30,500,000 tuberose cut flowers were produced from 119 ha of cultivation area (Edrisi and Mirzaei, 2017). Among ornamental plants, the family Agavaceae is compatible with warm and dry climate and grows well in these conditions (Barba-Gonzalez *et al*., 2012).

Most of this family's plants have leaf rosette and are rhizomatous and resistant to water stress. The reaction of tuberose plants to water deficits stress is uncertain, though. Due to the cultivation of tuberose in Khuzestan, Isfahan, Markazi and Tehran provinces in Iran (Shoor *et al*., 2005) and lack of studies on water stress and drought resistance mechanisms in this plant, the present study was carried out to evaluate the physiological characteristics (photosynthesis rate, stomatal resistance, transpiration rate, antioxidant enzymes activity, relative water content, electrolyte leak-

age, etc.) of tuberose cultivars under water deficit stress conditions.

MATERIALS AND METHODS

Experimental design and treatments

The research was carried out at a greenhouse in Ornamental Plants Research Center (longitude 35° 27' 30" E. and latitude 33° 54' 30" N.) located in Mahallat County, Markazi Province of Iran, and at the Laboratory of Horticultural Sciences in the Faculty of Agriculture, Lorestan University. Pots were arranged in factorial based on a complete randomized design (CRD) with three replications; each replication had four plants. The bulbs of tuberose (two cultivars including a double-flower and a single-flower cultivar) were provided by a commercial grower in Mahallat County, Iran (an average diameter of 2.8-3.7 cm). The bulbs were planted in pots (a diameter of 260 mm and a depth of 270 mm) in June 2016. The physical and chemical traits of the experimental soil are shown in Table 1.

Table 1. Some physical and chemical characteristics of the pot soils.

FC (%)	PWP (%)	pH	EC (dSm^{-1})	Sand (%)	Silt (%)	Clay (%)
19.55	13.16	7.51	0.4	51.35	22.09	26.56

FC: Field capacity, PWP: Permanent wilting point, EC: Electrical conductivity.

The field capacity of the soil and permanent wilting point (PWP) were measured by a pressure–plate apparatus. Before planting, the bulbs of the tuberoses were soaked in 0.2% benomyl fungicide (benlate 50% WP) to get disinfected from fungal pathogens for 30 min. The pots were irrigated at three-day intervals and the plants were allowed to establish for 21 days (at the greenhouse under natural light at 20-30C and relative humidity of 55%) before the initiation of water deficit treatments. Afterwards, the plants were exposed to water stress up to their flowering stage: no stress (W_1 = 90% of FC), moderate stress (W_2 = 75% of FC), and severe stress (W_3 = 60% of FC). Throughout the 70-day cultivation period, the moisture level in the growth media was controlled by daily weighing as per the procedure of Yadav *et al.* (2013). Briefly, the percentage of water in the potting media was determined at 24 h (the gravimetric water was allowed to drain) after the pots were watered using a 20 g soil sample collected from randomly chosen pots and oven-dried at 100C and then reweighed. The percentage of soil water content was calculated using the following formula (Yadav *et al*., 2013):

$$\text{Soil water content (\%)} = (SW-DW) / SW$$

where SW denotes the weight of saturated soil and DW represents the weight of dry soil.

Water leaching from pots was captured in a dish placed under each pot and returned to the soil before the addition of any new water. Physiological traits included relative water content (RWC) and electrolyte leakage (EL). At the flowering stage, fresh leaves were sampled for the measurement of RWC based on Lu *et al*. (2009) and of ion leakage based on Promyou *et al*. (2012).

Relative water content

For the measurement of RWC, fresh leaves were weighed (Wf) and then soaked in water overnight until their weight reached a plateau. The water-saturated leaves were weighed again (Ws) and then dried for 24 h at 80°C to determine their dry weight (Wd). Ultimately, RWC was calculated by the following formula:

$$RWC\ \% = (Wf - Wd) / (Ws - Wd) \times 100$$

Electrolyte leakage

To measure electrolyte or ion leakage, ten discs (15 mm diameter) were excised from the leaves with a cork borer to be rinsed with distilled water, immersed in 30 ml of 0.4 M mannitol solution, and shaken at 100 cycles per min at ambient temperature. After shaking for 3 h, the conductivity of the solution was measured with a conductivity meter (R_1= initial electrolyte leakage). Maximum electrolyte leakage was achieved by autoclaving the samples at 121°C for 1 h and then cooling them down to room temperature (R_2 = final electrolyte leakage). The percentage of ion leakage was calculated using the following equation (Promyou *et al*., 2012):

$$EL\ \% = R_1/R_2 \times 100$$

Chlorophyll and carotenoids contents

The chlorophyll was extracted by the method of Lichtenthaler (1987). The optical density (OD) of the extracted chlorophyll was measured at 622, 645, and 470 nm by using a spectrophotometer UV-1800. Total chlorophyll (chlorophyll a and b) and carotenoid contents were calculated by the following formulae:

Chlorophyll a ($\mu g\ g^{-1}$ FW) = 11.24 ($OD_{661.6}$) - 2.04 ($OD_{644.8}$)
Chlorophyll b ($\mu g\ g^{-1}$ FW) = 20.13 ($OD_{644.8}$) - 4.19 ($OD_{661.6}$)
Total chlorophyll ($\mu g\ g^{-1}$ FW) = Chlorophyll a + Chlorophyll b = 7.05($OD_{661.6}$) +18.09($OD_{664.8}$)
Carotenoids = (1000× OD_{470} -1.9× chlorophyll a - 63.14 × chlorophyll b)/214

Proline determination

Proline was determined following Bates *et al*. (1973). Briefly, 0.5 g of leaf samples were ground in 10 ml of sulphosalicylic acid and the resulted mixture was centrifuged at 15,000 g for 10 min at 4°C; 2 ml of filtrate was mixed with 2 ml of acid-ninhydrin and 2 ml of glacial acetic acid in a test tube. The mixture was incubated in a water bath for 60 min at 100°C and then immediately cooled with ice. To each tube, 4 ml of toluene was added into the reaction mixture after the solution was vortexed for 20 seconds. The absorbance of the organic phase was recorded at 520 nm. The results were compared with a standard curve of proline and the concentration was expressed in $\mu mol\ g^{-1}$ leaf fresh weight (FW).

Determination of lipid peroxidation

Lipid peroxidation was estimated by measuring the formation of malondialdehyde (MDA) with 2-thiobarbituric acid (TBA) according to Wang *et al.* (2009) with some modifications. Fresh leaf samples (0.1 g) were ground with 5 mL 0.5% TBA in 20% trichloroacetic acid (TCA) using a mortar and pestle. The resulted mixture was heated at 100℃ for 15 min. After cooling in ice, the mixture was centrifuged at 4,000 rpm min^{-1} for 10 min. The absorbance of the supernatant was measured at 450, 532, and 600 nm. The MDA content was calculated on a fresh weight basis as follows:

$$MDA\ (\mu mol\ g^{-1}\ FW) = 6.45\ (OD_{532}\text{-}OD_{600}) - 0.56\ (OD_{450})$$

Antioxidant enzymes activity

The activity of SOD was determined according to the method of Giannopolitis and Ries (1977) with some modifications. The reaction solution (1 mL) contained 50 mM of phosphate

buffer (pH = 7), 0.21 mM of riboflavin, 13 mM of methionine, 0.1 mM of EDTA, 75 µM of nitro blue tetrazolium (NBT), and 30 µL of extracted enzyme solution. A solution with no enzyme was used as the control. The test tubes were irradiated under fluorescent lights at 46 W for 15 min. The absorbance of each solution was measured at 560 nm using a spectrophotometer. One unit of enzyme activity was defined as the amount of enzyme that would inhibit 50 percent of NBT photoreduction.

The activity of CAT was determined based on the oxidation of H_2O_2 as per the method of Chance and Maehly (1955) with some modifications. The reaction solution (0.4 mL) contained 25 mM of phosphate buffer (pH=7), 0.3 mL of H_2O_2, and 30 µL of extracted enzyme solution. The reaction was initiated by adding the enzyme solution. The changes in absorbance at 240 nm were read every 10 s for 60 s using a spectrophotometer. One unit of CAT activity was defined as the absorbance change of 0.01 units per minute.

Ascorbate peroxidase activity was determined based on the oxidation of ascorbate using a modified version of the method of Nakano and Asada (1981). The reaction solution (1.5 mL) was composed of 100 mM of phosphate buffer (pH=7), 0.2 EDTA, 3 µL of H_2O_2, 1 mM of ascorbic acid, and 50 µL of extracted enzyme solution. The reaction was initiated by adding the enzyme extract. The changes in absorbance at 290 nm were read every 10 s for 60 s using a spectrophotometer. One unit of APX activity was defined as an absorbance change of 0.01 per min.

Leaf gas exchange measurements

Gas exchange (photosynthesis rate, transpiration rate, and stomatal resistance) of fully expanded leaves of two cultivars of tuberose were measured at the flowering stage with an ADC LCA-4 infrared gas analyzer (IRGA) equipped with a Leaf Microclimate Control System (ADC Hoddesdon Ltd., UK) at 22°C. The measurements were made between 8:00 a.m. and 10:00 a.m. in the greenhouse where the plants were kept during the experiment. Leaf area was 6.25 cm^2. The measurements were performed in photosynthesis active radiation 850 µmol m^{-2} s^{-1} (Wang *et al*., 2014).

Statistical analysis

Analysis of variance (ANOVA) was carried out with SAS 9.1 and GraphPad Prism 8 software packages. Differences of means were assessed by the LSD test with differences considered significant at $P < 0.05$.

RESULTS

Relative water content (RWC) and electrolyte leakage (EL)

The results showed that EL and RWC in the leaves of the tuberose plants were highly affected by water deficit stress (Table 2). Water deficit stress significantly increased EL in the leaves of tuberose plants (Tables 3 and 4).

Table 2. Analysis of variance for the traits of the tuberose cultivars.

S.o.V	df	MS						
		EL	RWC	A	E	r_s	Total chl.	Carotenoid
Treatment (T)	2	84.14***	172.35***	10.55***	0.10***	839.83***	43.54***	2.17***
Cultivar (C)	1	28.00***	84.15**	0.96*	0.025*	58.12*	3.99*	0.85***
T×C	2	3.67*	9.15^{ns}	0.81*	0.003^{ns}	1.95^{ns}	0.011^{ns}	0.089^{ns}
Error	12	0.78	6.47	0.18	0.005	9.93	0.66	0.035
CV (%)	-	4.71	3.23	13.62	9.17	5.77	7.43	10.55

*, ** and ^{ns}: Significant at $P < 0.05$, $P < 0.01$ and insignificant, respectively. EL: Electrolyte leakage, RWC: Relative water content, A: Photosynthesis rate, E: Transpiration rate, r_S: Stomatal resistance, Chl: Total chlorophyll.

Table 2. Continued.

SoV	df	MS				
		SOD	APX	CAT	MDA	Proline
Treatment (T)	2	392.39***	0.0097***	0.089***	0.29***	0.002***
Cultivar (C)	1	8.09*	0.0015***	0.031**	0.017*	0.0004*
T×C	2	0.27^{ns}	0.0004**	0.0007^{ns}	0.002^{ns}	0.00007^{ns}
Error	12	1.39	0.00003	0.002	0.004	0.00005
CV (%)	-	4.07	2.71	9.36	10.32	8.10

*, ** and ^{ns}: Significant at $P < 0.05$, $P < 0.01$ and insignificant, respectively. SOD: Superoxide dismutase, APX: Ascorbate peroxidase, CAT: Catalase, MDA: Malondialdehyde.

Table 3. The variations of the physiological characteristics among tuberose cultivars.

Characteristics	Treatments		LSD
	Double	Single	
EL (%)	20.00^{a}	17.51^{b}	0.91
RWC (%)	76.50^{b}	80.82^{a}	2.67
A (µmol m^{-2} s^{-1})	2.93^{b}	3.39^{a}	0.44
E (mmol m^{-2} s^{-1})	0.79^{a}	0.71^{b}	0.07
r_S (mol m^{-2} s^{-1})	52.74^{b}	56.34^{a}	3.24
Total chl (µg g^{-1} FW)	10.49^{b}	11.44^{a}	0.84
Carotenoid (µg g^{-1} FW)	1.98^{a}	1.55^{b}	0.19
SOD (µmol g^{-1} FW)	28.33^{b}	29.67^{a}	1.21
APX (µmol min g^{-1} FW)	0.1814^{b}	0.1997^{a}	0.0053
CAT (µmol min g^{-1} FW)	0.5399^{a}	0.4570^{b}	0.0479
MDA (µmol g^{-1})	0.6148^{a}	0.5528^{b}	0.0619
Proline (µmol g^{-1} FW)	0.0795^{b}	0.0887^{a}	0.007

In each lines, means with the similar letter(s) are not significantly different ($P < 0.05$) using the LSD test. Double and single treatments included tuberose cultivars including double-flower and single-flower, respectively. EL: Electrolyte leakage, RWC: Relative water content, A: Photosynthesis rate, E: Transpiration rate, r_S: Stomatal resistance, Chl: Total chlorophyll, SOD: Superoxide dismutase, APX: Ascorbate peroxidase, CAT: Catalase, MDA: Malondialdehyde.

Table 4. Effects of water deficit stress on physiological characteristics of tuberose cultivars.

Characteristics	Treatments			LSD
	W_1	W_2	W_3	
EL (%)	15.17^{c}	18.46^{b}	22.64^{a}	1.11
RWC (%)	83.19^{a}	80.04^{a}	72.74^{b}	3.20
A (µmol m^{-2} s^{-1})	4.58^{a}	2.97^{b}	1.94^{c}	0.54
E (mmol m^{-2} s^{-1})	0.88^{a}	0.74^{b}	0.62^{c}	0.08
r_s (mol m^{-2} s^{-1})	41.89^{c}	56.40^{b}	65.33^{a}	3.96
Total chl (µg g^{-1} FW)	13.94^{a}	10.26^{b}	8.70^{c}	1.02
Carotenoid (µg g^{-1} FW)	1.29^{c}	2.44^{a}	1.56^{b}	0.23
SOD (µmol g^{-1} FW)	35.00^{a}	32.2^{b}	19.81^{c}	1.49
APX (µmol min g^{-1} FW)	0.1570^{c}	0.1795^{b}	0.2352^{a}	0.0065
CAT (µmol min g^{-1} FW)	0.3817^{c}	0.4882^{b}	0.6255^{a}	0.0587
MDA (µmol g^{-1})	0.3585^{c}	0.5970^{b}	0.7958^{a}	0.0758
Proline (µmol g^{-1} FW)	0.0660^{c}	0.0820^{b}	0.1043^{a}	0.0086

In each lines, means with the similar letter(s) are not significantly different ($P < 0.05$) using the LSD test. W_1, W_2 and W_3 treatments consist of water deficit stress, 90% FC, 75% FC and 60% FC, respectively. EL: Electrolyte leakage, RWC: Relative water content, A: Photosynthesis rate, E: Transpiration rate, r_s: Stomatal resistance, Chl: Total chlorophyll, SOD: Superoxide dismutase, APX: Ascorbate peroxidase, CAT: Catalase, MDA: Malondialdehyde.

The leaves of the double-flower cultivar had significantly higher EL than the leaves of the single-flower cultivar regardless of water treatments. Water stress significantly decreased RWC in the leaves of tuberose plants. However, no significant differences were observed between the W_1 and W_2 water stress levels. EL showed a significant correlation with most physiological traits including RWC ($r=-0.896^{***}$), r_s ($r=0.733^{***}$), APX ($r=0.703^{**}$), CAT ($r=0.908^{***}$), proline ($r=0.678^{**}$), MDA ($r=0.895^{***}$), A ($r=-0.855^{***}$), E ($r=-0.661^{**}$), total chl ($r=-0.890^{***}$), and SOD ($r=-0.873^{***}$). Similalry, RWC showed a significant correlation with most physiological traits, i.e. EL ($r=-0.896^{***}$), r_s ($r=-0.644^{**}$), APX ($r=-0.596^{*}$), CAT ($r=-0.894^{***}$), proline ($r=-0.571^{*}$), MDA ($r=-0.757^{***}$), A ($r=0.774^{***}$), E ($r=0.639^{**}$), total chl ($r=0.766^{***}$), and SOD ($r=0.851^{***}$) (Table 5). RWC was significantly higher in the leaves of the single-flower cultivar that in the leaves of the double-flower cultivar regardless of water treatments (Figs. 1 and 2).

Leaf gas exchange

Water stress was affected by photosynthesis rate (A). The photosynthesis rate of tuberose subjected to water stress was significantly different among the cultivars. The photosynthesis rate was significantly decreased in plants grown under the W_3 conditions. Responses from the double-flower cultivar, however, exhibited a greater decrease in photosynthesis rate than the single-flower cultivar under water stress. The single-flower cultivar had a higher amount of photosynthesis rate (3.39) than the double-flower cultivar (2.93) in water stress conditions. Significant differences were observed among W_1, W_2 and W_3 levels in water stress conditions (Tables 2, 3 and 4).

The effect of water stress on transpiration rate (E) varied between cultivars and water stress treatments. The transpiration rate was decreased with the increase in water deficit stress. A significant increase was observed in the W_3 treatment. A significant decrease in the transpiration rate was observed in the single-flower cultivar versus the double-flower cultivar. With the increase in water stress treatment, stomatal resistance continuously changed. It was also observed that water stress increased stomatal resistance in the single-flower versus the double-flower cultivar. The

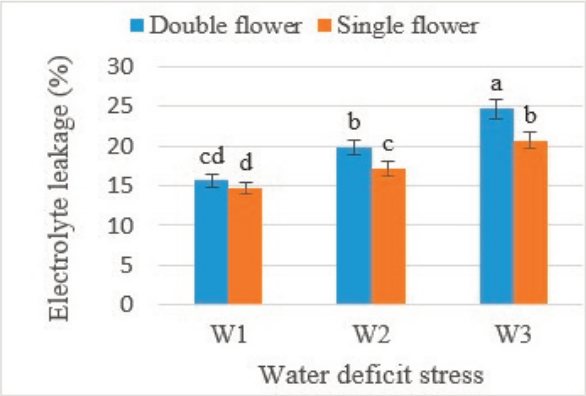

Fig. 1. Effects of water deficit stress on electrolyte leakage in tuberose cultivars.

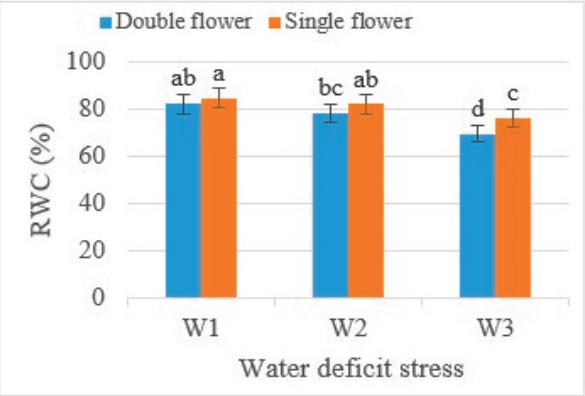

Fig. 2. Effects of water deficit stress on RWC in tuberose cultivars.

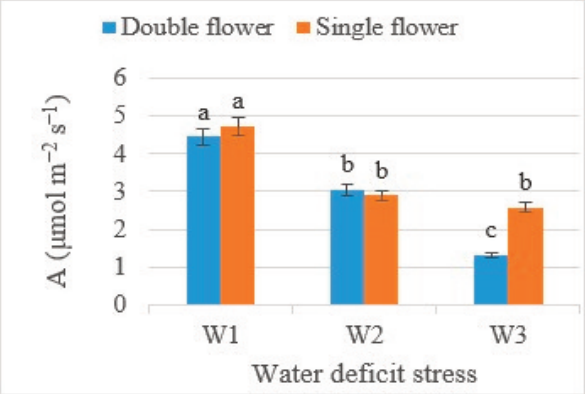

Fig. 3. Effects of water deficit stress on A (photosynthesis rate) in tuberose cultivars.

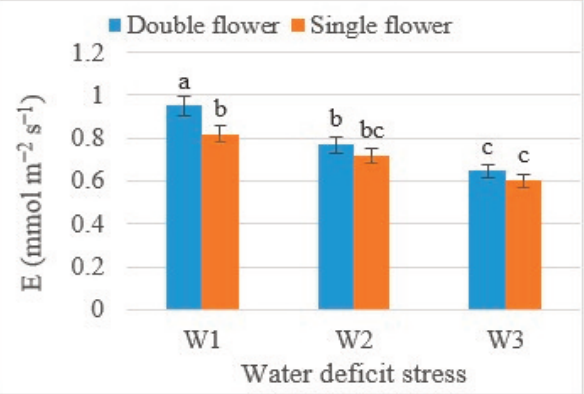

Fig. 4. Effects of water deficit stress on E (transpiration rate) in tuberose cultivars.

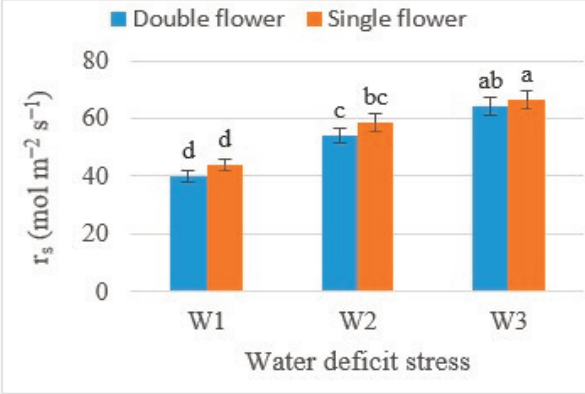

Fig. 5. Effects of water deficit stress on rs (stomatal resistance) in tuberose cultivars.

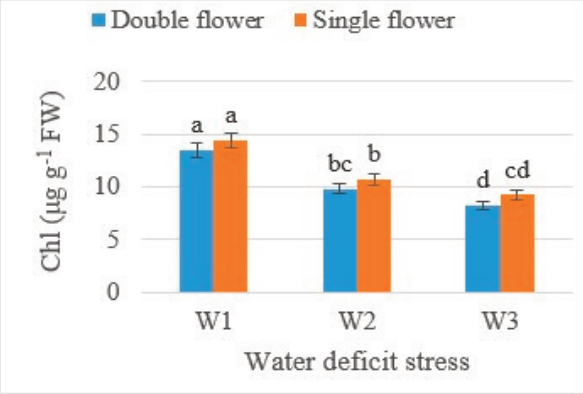

Fig. 6. Effects of water deficit stress on total chlorophyll in tuberose cultivars.

highest average stomatal resistance (56.34) was recorded in the single-flower cultivar while it was 52.74 in the double-flower cultivar(Figs. 3, 4 and 5). Leaf gas exchange (A, E, and r_s) showed a significant correlation with most physiological characteristics such as EL, APX, CAT, proline, MDA, RWC, Chl, and SOD (Table 5).

Chlorophyll and carotenoid contents

Water stress significantly decreased the content of total Chl in the leaves of tuberose plants (Tables 2, 3 and 4). The total Chl content in the leaves of the single-flower cultivar was significantly higher than that in the double-flower cultivar regardless of water treatments (Fig. 6). Water stress significantly increased the content of carotenoids in the leaves of tuberose plants. The carotenoids content in the leaves of the double-flower cultivar was significantly higher than that in the single-flower cultivar regardless of water treatments (Fig. 7). Total Chl showed a significant correlation with most physiological characteristics including EL ($r=-0.890^{***}$), RWC ($r=0.766^{***}$), A ($r=0.849^{***}$), E ($r=0.761^{***}$), r_s ($r=-0.833^{***}$), SOD ($r=0.821^{***}$), APX ($r=-0.763^{***}$), CAT ($r=-0.850^{***}$), MDA ($r=-0.871^{***}$), and proline ($r=-0.771^{***}$). Carotenoid showed an insignificant correlation with the physiological traits (Table 5).

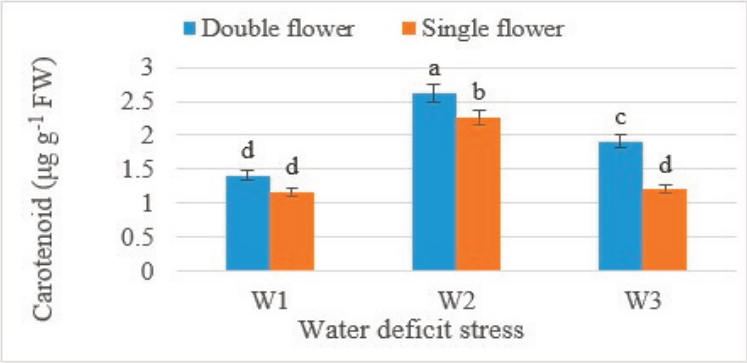

Fig. 7. Effects of water deficit stress on carotenoid in tuberose cultivars.

Table 5. Correlation coefficients of the studied traits in tuberose cultivars.

	EL	RWC	A	E	r_s	Chl	Car	SOD	APX	CAT	MDA	Proline
EL	1.000											
RWC	-0.896^{***}	1.000										
A	-0.855^{***}	0.774^{***}	1.000									
E	-0.661^{**}	0.639^{**}	0.674^{**}	1.000								
r_s	0.733^{***}	-0.644^{**}	-0.880^{***}	-0.847^{***}	1.000							
Chl	-0.890^{***}	0.766^{***}	0.849^{***}	0.761^{***}	-0.833^{***}	1.000						
Car	0.347^{ns}	-0.251^{ns}	-0.408^{ns}	-0.251^{ns}	0.341^{ns}	-0.457^{ns}	1.000					
SOD	-0.873^{***}	0.851^{***}	0.816^{***}	0.756^{***}	-0.796^{***}	0.821^{***}	-0.003^{ns}	1.000				
APX	0.703^{**}	-0.596^{*}	-0.735^{**}	-0.787^{***}	0.849^{***}	-0.763^{***}	-0.073^{ns}	-0.883^{***}	1.000			
CAT	0.908^{***}	-0.894^{***}	$-.0849^{***}$	-0.706^{**}	0.770^{***}	-0.850^{***}	0.331^{ns}	-0.891^{***}	0.704^{**}	1.000		
MDA	0.895^{***}	-0.757^{***}	-0.921^{***}	-0.672^{**}	0.855^{***}	-0.871^{***}	0.384^{ns}	-0.851^{***}	0.785^{***}	0.860^{***}	1.000	
Proline	0.678^{**}	-0.571^{*}	-0.823^{***}	-0.702^{**}	0.892^{***}	-0.771^{***}	0.054^{ns}	-0.804^{***}	0.912^{***}	0.719^{**}	0.807^{***}	1.000

*, ** and ^{ns}: Significant at $P < 0.05$, $P < 0.01$ and insignificant, respectively. EL: Electrolyte leakage, RWC: Relative water content, A: Photosynthesis rate, E: Transpiration rate, r_s: Stomatal resistance, Chl: Total chlorophyll, SOD: Superoxide dismutase, APX: Ascorbate peroxidase, CAT: Catalase, MDA: Malondialdehyde.

Antioxidant enzymes

Water stress caused a significant increase in APX, CAT and MDA activities in the leaves of tuberose plants (Table 2). The SOD activity in the tuberose leaves was decreased by increasing the water stress level. The APX and SOD activities in the leaves of the single-flower cultivar were significantly higher than those in the double-flower cultivar regardless of water treatments. The CAT and MDA activities in the leaves of the double-flower cultivar were significantly higher than those in the single-flower cultivar regardless of water treatments (Tables 3 and 4). The catalase activity was increased under water stress conditions in both double- and single-flower cultivars (Figs. 8, 9, 10 and 11).

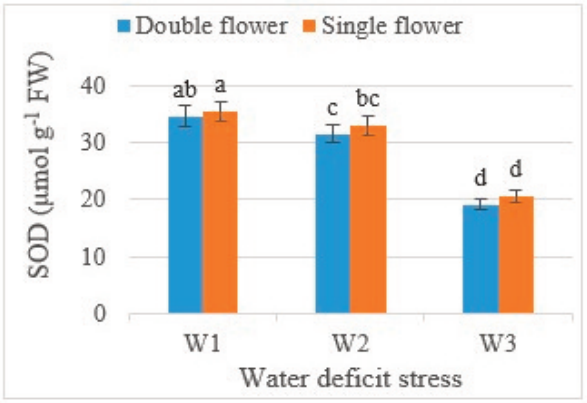

Fig. 8. Effects of water deficit stress on SOD in tuberose cultivars.

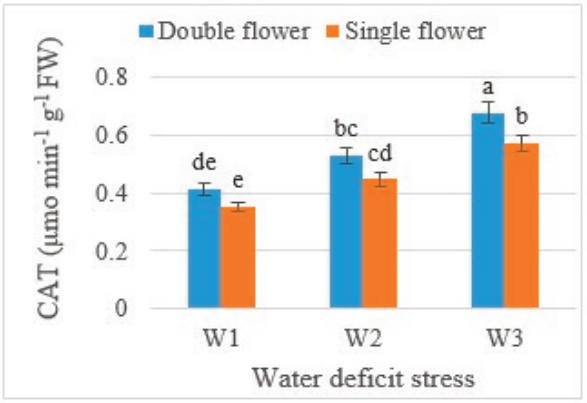

Fig. 9. Effects of water deficit stress on CAT activity in tuberose cultivars.

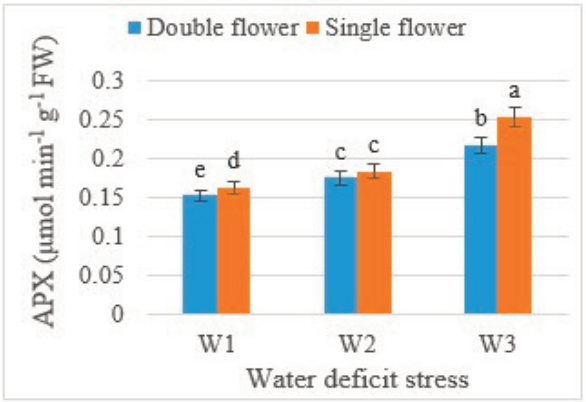

Fig. 10. Effects of water deficit stress on APX activity in tuberose cultivars.

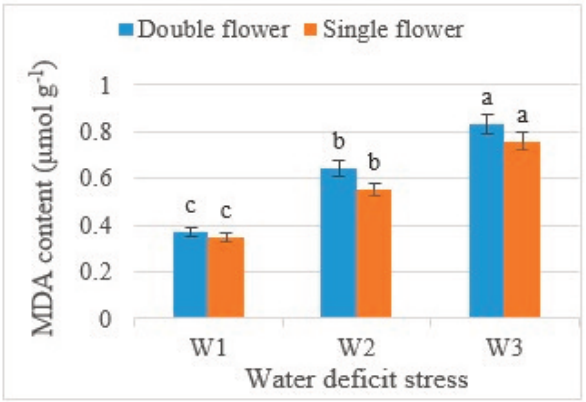

Fig. 11. Effects of water deficit stress on MDA content in tuberose cultivars.

Proline

The proline content in tuberose leaves was increased with the increase in the water stress level. Moreover, the proline content in the leaves of the single-flower cultivar was significantly higher than that in the double-flower cultivar (Fig. 12). Proline showed a significant correlation with most physiological traits such as the EL ($r=0.678^{**}$), RWC ($r=-0.571^{*}$), A ($r=-0.823^{***}$), E ($r=-0.702^{**}$), r_s ($r=0.892^{***}$), Chl (-0.771^{***}), SOD ($r=-0.804^{***}$), APX ($r=0.912^{***}$), CAT ($r=0.719^{**}$) and MDA ($r=0.807^{***}$) (Table 5).

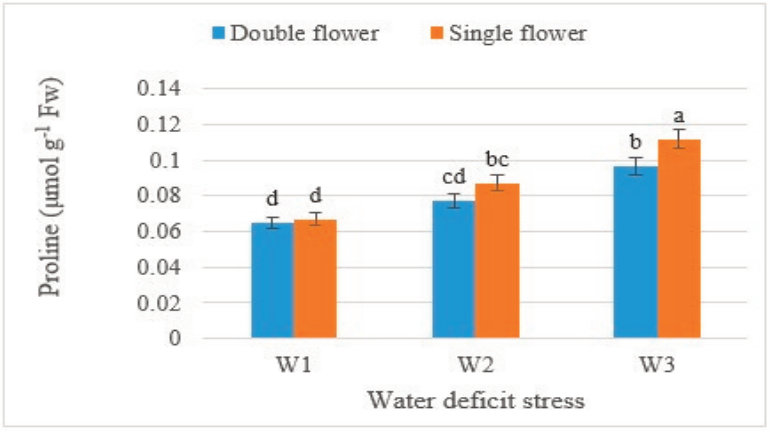

Fig. 12. Effects of water deficit stress on proline in tuberose cultivars.

DISCUSSION

The results presented here indicate that water stress influences the physiological and biochemical traits of tuberose. Increasing water stress level caused a decrease in RWC, A, E, SOD, and total chl. and an increase in EL, r_s, carotenoids, APX, CAT, MDA, and proline. These results are consistent with other studies on physiological, biochemical traits and water stress (Lu *et al*., 2009; Wang *et al*., 2009; Riaz *et al*., 2010; Konnerup and Brix, 2010; Anjum *et al*., 2011b; Yadav *et al*., 2013; de Lima *et al*., 2015).

The higher RWC is associated with water stress resistance and has also been proposed as a more valuable indicator of plant status (Basu *et al.*, 2010). The results of this study showed that the single-flower cultivar maintained a relatively higher leaf RWC than the double-flower cultivar (Tables 3 and 4). This could be the reason (as the single-flower cultivar is closer to the native and unbred genotype) for the higher tolerance to water stress in the single-flower cultivar. Leaf RWC is probably the most appropriate measure of the plant water status as it decreases as the water deficit stress progresses and causes changes in the cell membrane and thus increases electrolyte leakage from the cells. With stomata closure and the reduction of transpiration, water uptake towards the leaves decreases. The decrease in RWC is due to the reduction of leaf water potential and the loss of water uptake by roots under drought stress conditions (Soni and Abdin, 2017).

It was observed that drought stress increased EL and MDA in the double-flower cultivar as compared to the single-flower cultivar, which may be attributed to oxidative damage to the biological membranes (Hatamzadeh *et al*., 2015). This result is confirmed by other authors such as Mzabri *et al*. (2017) who studied the effect of drought stress on the growth and development of saffron (*Crocus sativus* L.) in eastern Morocco (Mzabri *et al*., 2017).

Increasing water stress caused an alteration in the photosynthesis rate. This increase under W_2 may be attributed to the Calvin photosynthetic cycle (C_3) metabolism under unstressed conditions, which is shifted to crassulacean acid Metabolism (CAM) in response to water stress (Taiz and Zeiger, 2010). Our results do not support those of Souza *et al*. (2004) who stated that the decrease in transpiration rate in water-affected plants was largely dependent on stomatal closure because it reduced internal CO_2 and photosynthetic rate (Souza *et al.,* 2004; Naidoo and Naidoo, 2018).

These results could be in agreement with those obtained by Mittler (2002), who suggested that the source of reducing energy for ROS scavenging during stress was accompanied by the suppression of the photosynthetic apparatus. In addition, they attributed the reduction in chlorophyll content to the suppression of specific enzymes that are responsible for the synthesis of photosyn-

thetic pigments (Murkute *et al*., 2006). The reduction in chlorophyll contents in response to water stress is mainly due to the stomatal closure induced by the ABA increase in cowpea plants and the transpiration rate (EL-Bassiouny, 1997).

The chlorophyll content is known as an index to evaluate the tolerance of plants to water stress; it is used to index high stability that determines that a plant has not been stressed by showing increased access to chlorophyll (Rivas *et al*., 2016). Water deficit stress increased ROS synthesis in leaves and caused chlorophyll degradation. The decrease in total chlorophyll in response to drought stress may affect nitrogen metabolism for the biosynthesis of compounds such as proline during osmotic adjustment. Increasing proline content inhibits glutamate (a precursor of chlorophyll and proline) which reduces its involvement in chlorophyll synthesis (Hosseinzadeh *et al*., 2016).

Photosynthesis is the primary process in plant biomass production and is one of the most sensitive physiological processes in response to environmental stresses. Consequently, the ability to maintain a reasonable rate of photosynthesis under stressful conditions can be a good indicator of a plant's adaptability (Niu *et al*., 2006). Photosynthetic pigments are important to plants mainly for light harvesting and production of reducing agents. Water stress can also alter the tissue concentration of chlorophylls and carotenoids. In plants, the mechanisms of drought stress tolerance might be associated with the increased activity of antioxidant enzymes such as APX, CAT, SOD, etc. (Farooq *et al*., 2009).

Under stressful conditions, the reduction of chlorophyll content may be associated with chlorophyll degradation or photooxidation of pigments (Anjum *et al.*, 2011a; Nadeem *et al*., 2014). Carotenoids, photosynthetic pigments, can scavenge oxygen radicals and lipid peroxy radicals and inhibit lipid peroxidation and superoxide generation under dehydrative forces (Sanchez-Rodriguez *et al*., 2010).

Increased SOD activity in the early stage of drought stress protects plants from oxidative damage. However, the decrease in SOD activity after prolonged drought stress indicates that the elimination of ROS by SOD is weakened (Fu and Huang, 2001).

Drought stress increases ROS synthesis in leaves and induces chlorophyll degradation (Anjum *et al*., 2011a). The total chlorophyll content significantly decreases under water stress. These results suggest the presence of genetic variability in pigment retention between the two cultivars under water stress conditions. It is suggested that the higher concentrations of catalase and ascorbate peroxidase might have removed the oxygen radicals and its product H_2O_2 induced by water stress (Moussa and Abdel-Aziz, 2008). It is known that plants have a well-organized defense system against ROS under stressful conditions and SOD constitutes the first line of defense via detoxification of superoxide radicals (Xu *et al*., 2015). Increasing the concentration of MDA under water stress indicates that drought stress can lead to the induction of membrane peroxidation by free oxygen species (Aghdam *et al*., 2015). A high content of proline enables plants to survive through the effects of water stress. Under stressful conditions, water is absorbed from the environment by lowering water potentials and the accumulation of compatible osmolytes *(*Kumar *et al.*, 2003).

CONCLUSION

The results of this experiment could change the attitude towards water consumption during the cultivation of tuberose. We conclude that water deficit stress W_3 treatment injures tuberose. The amount of water stress damage in tuberose is cultivar- dependent. The single-flower cultivar is more tolerant of water stress than the double-flower one. The higher photosynthesis rate under water stress conditions suggests that the single-flower cultivar has a high capacity for water absorption and mobilization to leaves.

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