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Original Article

Assessment of drought tolerance capacity of tuberose (*Polianthes tuberosa* L.) on the basis of various growth and physio–chemical indicators

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Abstract

Bulbous crops like tuberose (*Polianthes tuberosa* L.) needs abundant amount of water for its better growth and development as compared to other floricultural crops. A mismanagement of irrigation may lead to severe damages to the crop and huge economic loss. Thus, to understand basis of irrigation needs and its impact on tuberose plant, a pot experiment was performed. Drought tolerance capacity of tuberose was assessed by analyzing morphological, physiological, enzymatic and bio-chemical attributes of two tuberose cultivars (Mexican Single and Pearl Double) under different irrigation regimes. Plants were grown in the plastic pots arranged in completely randomized design (CRD). Seven irrigation intervals (2, 3, 4,5,6,7 and 8 days; viz. T_2-T_7 respectively; T_1 = control) were applied. Pots were placed in growth chamber with 30/25°C day/night temperatures and 50±5 % relative humidity. Results revealed that growth parameters in tuberose plant in both cultivars showed better performance under minimum irrigation interval (2 days) followed by 3 days and 5 days interval. Drought caused significant decreases in height of flowering stem (HFS; T_2: 20 cm; T_8: 10 cm), leaf area (LA; T_2: 53.3cm^2; T_8: 16.9cm^2), plant height (PH; T_2: 27.6; T_8: 12.74), fresh weight of flowering stem (FWFS;T_2: 37.5;T_8: 12.6), relative water content (RWC), photosynthesis rate (A), transpiration rate (E), stomatal conductance (SC) and chlorophyll contents (Chl) and increases in leaf water potential (LWP), water use efficiency (WUE), antioxidant enzyme activities (CAT, POD, SOD) and biochemical traits (Pro and Gly). However, enzymes activities like CAT, POD, and SOD; proline and glycinebetaine in the leaves of cultivar 'Pearl Double' were observed significantly higher than those in the leaves of 'Mexican Single' cultivar, regardless of irrigation intervals (water treatments). The results indicated that the growth performance of 'Mexican Single' cultivar was better than the 'Pearl Double' cultivar in drought conditions.

Keywords: Drought, Tuberose, Relative water content, Antioxidants, Photosynthesis

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Introduction

Today water scarcity is the most important and critical factor which reduces the yield and production of all agriculture crops not only in the arid but also in semi-arid areas of the world (Kumar et al., 2012). The decrease in rainfalls, groundwater tables and increase in environment temperature due to global warming has resulted in the very costly water availability for irrigation purposes all over the world. Total area of Pakistan is 79.6 million hectare of which 70% is arid to semi-arid and only 22 million hectare (27.6%) consists of cultivated lands. Its average annual rainfall is below 240 mm. Southern and northern Khyber Pakhtunkhwa range from semi-arid to humid. Sindh is mostly arid while Punjab and Balochistan are arid–semi arid to humid (Farooq et al., 2007).

Water shortage inhibits extension in growth and damages most of plants severely in semi-arid as well as arid areas of the world (Riaz et al., 2013). Photosynthesis and respiration are the two main processes which are reduced due to decreased supply of water (Niu et al., 2006). Plants start to accumulate osmolytes like proline and reduce photosynthesis and other growth processes under water stress conditions and in consequence of this, they start to produce reactive oxygen species (ROS) which ultimately cause damages to cell by degrading proteins, nucleic acids and lipids (Anjum et al., 2011).

Plants protect the destructive damages of ROS by different defensive mechanisms including enzymatic antioxidants (catalase, peroxidase and superoxide dismutase) and non-enzymatic antioxidants (glutathione, ascorbate and carotenoids) at cellular level. The important mechanisms include reduced water loss, increased water absorption through deep roots and succulent leaves which can reduce water loss by transpiration. The osmolytes such as proline, glycinebetaine, amino acids and some other organic acids play vital role to sustain cellular functions in water deficit conditions (Farooq et al., 2009).

So now there is a need for developing and adopting such techniques and methods such as production of drought tolerant species, use of growth regulators which can overcome the shortage of water for ornamental species particularly bulbous plants. The introduction of such plant species which can tolerate drought and need less amount of water for growth functions is an emerging approach in this regard (Riaz et al., 2013).

Tuberose is a bulbous ornamental crop which is cultivated due to its demand as cut flower, fragrant flowers and source of essential oil. It is native of Mexico and belongs to family *agavaceae* which comprises of such plant species which like warm and dry climatic conditions for their growth and development and also can resist water stress& deficit conditions. From Mexico, it was distributed to Europe and other eastern countries (Barba-Gonzalez et al., 2012).

Effect of irrigation intervals on morphological, physiological, enzymatic and bio-chemical attributes in tuberose (*Polianthes tuberosa* L.) under water deficit and stress conditions has not been investigated too much as compared to other ornamental crops, hence the behavior of tuberose cultivars to water stress is not certainly revealed yet. Tuberose is cultivated in Pattoki and Gujranwala cities of Punjab province in Pakistan on large scale. As the demand of cut flowers like gladiolus, rose, tuberose, marigold, gerberas and carnation has emerged rapidly in big cities in Pakistan, hence there is need of research studies on drought resistance and water stress tolerance potential in this crop (Usman and Ashfaq, 2013). Keeping in view such needs, the present research study was conducted to find out the effects of irrigation interval on growth, leaf water relations, physiological, enzymatic and bio-chemical characteristics of two tuberose cultivars 'Mexican Single' and 'Pearl Double' under water deficit stress conditions in Sargodha, Punjab, Pakistan.

Material and Methods

Treatments and experimental design

The present research was carried out in the Laboratory of the Department of Horticulture, College of Agriculture, University of Sargodha, Pakistan, during the year 2018. Bulbs (average 3-4 cm diameter) of cultivars 'Mexican Single and Pearl Double' were purchased from Lahore city, Punjab. The bulbs were sterilized with 0.2 % benomyl fungicide and planted in 30× 20 cm (height × diameter) plastic pots containing 3kg of soil in each pot as growth media. The treatments comprised of seven irrigation intervals; 2 (T_1), 3 (T_2), 4 (T_3), 5 (T_4), 6 (T_5), 7 (T_6) and 8 (T_7) days interval. Before the start of applying irrigation interval treatments, the pots were irrigated after three days intervals for four weeks to make them established.

The pots were then placed in growth chambers at 30/25°C (day/night), 50±5 % relative humidity and 400 µmol m^{-2} s^{-1} photosynthetic active radiation (PAR) light. The various growth, leaf water relations, gas exchange, enzymatic and biochemical attributes were measured. The research was conducted with CRD having factorial arrangement and consisting of two factors (cultivar, irrigation interval) along with four replications.

The parameters including height of flowering stem (HFS), flowering stem diameter (FSD), plant height (PH) and leaf area (LA) were measured. Flowering stems were harvested and weighed on digital balance as fresh weight of flowering stem (FWFS). These stalks were then oven dried for two days at 70°C and dry weight of flowering stem (DWFS) was measured. Similarly, shoot fresh weight (SHFW), and shoot dry weight (SHDW) were recorded.

Measurement of growth parameters

Five random plants from each treatment were selected while data collecting at different maturity stages after planting. Mean of each data was statistically analyzed. The length of flowering stem was measured in centimeter (cm) by keeping lower end of the measuring rod touching the base of bottom floret up to the upper floret of the spike, when two basal pairs of flowers had opened. Heights of 05 randomly selected flowering stalks from every replication were measured in cm. The mean of each replication was analyzed. Vernier caliper was used to measure the flowering stem diameter. Harvested flowering stalks weighed by using digital electric balance as fresh weight of flowering stem and were recorded in grams. Average of fresh biomass per replicate was noted. Plants used for fresh biomass were oven dried for two days at 70°C (Korl Kolb112 SL, Germany) and dry biomass was noted on electric balance in grams as dry weight of flowering stem. Since tuberose is bulbous crop and does not possess the true stem, the length of tallest stem was taken as plant length starting from the lower part of stem to the upper point of its shoot and mean values for all replicates were computed. It was recorded by measuring tape in centimeters and was measured when plant was at full bloom stage. Six leaves were selected from each plant and two plants per replication. Leaf area meter (Ll-3100; LI-COR,) was used to measure the leaf area (Michael et al., 2002). Plants were uprooted from soil. After cleaning, shoots were separated and weighed as shoot fresh weight by digital electric balance grams. After shoot fresh weights, these shoots were kept in drying oven (Korl Kolb-112 SL, Germany) for 48 hours at 70°C in paper bags. Digital electric balance was used for shoot dry weight.

Leaf water relations

After sixty days of plants growth, two young leaves were cut from each replication/treatment and kept in the pressure chamber following the method of Scholander et al., (1965) and leaf water potential (LWP; Ψw) observations were recorded in the morning times before 12:00 am. Leaf osmotic potential (LOP; Ψπ), the leaves used for LWP were frozen (-20 °C) for seven days and leaf cell sap was extracted. The leaf sap (10 µL) was placed on vapour pressure osmometer chamber and leaf osmotic potential (LOP; Ψπ) was recorded. Leaf turgor potential (LTP; Ψp) was calculated by subtracting the LOP from LWP. For relative water content (RWC; %), the uppermost leaves were cut and weighed as fresh weight (FW); then same leaves were soaked in distilled water to saturate and their weight taken as turgid weight (TW); and at the end, dried at 70°C for overnight and the measurement of their weight was done as dry weight (DW). The formula used for relative water content (%) was: RWC % = FW – DW / TW – DW x 100 as described by De Pascale et al., (2003).

Physiological characteristics

Measurement of the readings of leaf gas exchange traits like photosynthesis rate (A), transpiration rate (E) and stomatal conductance (g_s) were performed on an infrared gas analyzer (IRGA) in two hours interval 10: 00 am to 12: 00 am day time selecting two youngest leaves per treatment per replication as described by Shahid et al., (2011). Determination of water use efficiency was found using the equation; WUE = *A/E*. *C*hlorophyll contents were extracted by method of Lichtenthaler (1987) by crushing the leaves with mortar and pestle and placing the extract overnight in 80 % acetone at-40°C. The supernatant absorbance (optical density) of chlorophyll extract was measured at 645, 663 and 480 nm in spectrophotometer.

The formulae used were as below:

Chl (a) = [12.7(OD663) - 2.69(OD 645)] X Volume/ 1000 X weight
Chl (b) = [22.9(OD 645) - 4.68 (OD663)] X Volume / 1000 X weight
Total Chl = Chl (a) + Chl (b)
Total Chl = [20.2 (OD 645) + 8.02 (OD 663)] × V/1000 × W
Where V denotes the volume of acetone used (ml of acetone used) and W for weight of the leaf sample (mg leaf tissue); OD is optical density of extracted chlorophyll.

Antioxidant enzyme activity

The SOD activity was estimated with Giannopolitis and Ries (1977) method. A reaction solution (3mL) was prepared with 50 mM phosphate buffer, 50 mM of nitro blue tetrazolium (NBT), 1.3 mM riboflavin, 13 nM methionine, 75 mM EDTA and 50 mL enzyme extract solution. The control solution had no enzyme. The reaction solution were irradiated below the fluorescent bulbs of 40 W for 15 minutes and the absorbance of each reaction solution sample was noted at 560 nm in spectrophotometer. The enzyme amount that declined 50 % of NBT photo reduction was considered as SOD activity. The CAT and POD activities were determined by the method of Chance and Maehly (1955). The reaction solution (3mL) was prepared by dissolving 50 mM phosphate buffer and 5 mM H_2O_2 (1mL) with 0.1 mL extract solution. Changes in absorbance were measured after 20 seconds interval at 240 nm in spectrophotometer. Absorbance change of 0.01 units per minute was defined as one unit CAT activity. For the determination of POD activity, the reaction solution (3mL) was prepared by dissolving 50 mM phosphate buffer (pH 5.0), 20 mM guaiacol and 40 mM $H_2O_2$with 0.1 mL of enzyme extract solution. The differences in the absorbance of reaction solution samples were noted after every 20 seconds at 470 nm in spectrophotometer and the difference of 0.01 units per minute in absorbance was assigned to be one unit POD activity.

Biochemical parameters

Estimate of proline content was determined following the protocol of Bates et al., (1973). Leaf tissue sample (0.5 g) was ground in 10 mL of sulpho-salicyclic acid, centrifuged and 2 mL supernatant was mixed in 2 mL each of acidnin-hydrin and glacial acetic acid. The solution was heated at 100 °C for 1 hour. This solution was cooled and 4 mL toluene was added for proline extraction. The separation of aqueous state of solution was achieved and reading of absorbance was made by placing the solution samples at 520 nm in spectrophotometer using toluene as blank. A standard curve of proline was used to calculate proline as µmol g^{-1} leaf fresh weight (FW). For glycinebetaine measurement, 1g fresh leaf was homogenized in 10 mL distilled water and 5ml toluene water (0.5%) was added and incubated at 25°C for 24 h. The filtered extract was taken in flask and its volume was made upto 100 ml. A mixture solution of 1ml filtrate and 1ml of 2N HCl was prepared. Then an aliquot 0.5 ml from the previous extract was taken and 0.1 ml of potassium tri-iodide solution was added in it. It was then shaken in an ice bath for 90 min and then ice water (2 ml) was added along with 4ml of 1,2 dichloromethane. After stirring manually for some time, two layers were formed and clearly seen. The lower layer was used for taking reading. The absorbance/optical density (OD) was noted at 365nm in spectrophotometer according to the method of Grieve and Grattan (1983).

Statistical analysis

The experiment was carried out under complete randomized design (CRD) with two factor factorial arrangement Fisher's technique of statistical analysis of variance was used using statistical software statistic 8.1 and treatment means were grouped following significant difference (HSD, Tuckey test) and compared at 5% probability by LSD test (Steel et al., 1997).

Results

Growth parameters

Application of different irrigation intervals affected growth characteristics like height of flowering stem, flowering stem diameter, fresh and dry weight of flowering stem, plant height, leaf area, shoot fresh and dry weight significantly (Supplementary Table 1). These characters decreased consistently as the irrigation interval became longer indicating reduced growth of both the cultivars studied. Height of flowering stem (HFS) decreased significantly with the increase in irrigation intervals compared to frequent irrigation (Supplementary Table 1). However, in cultivar 'Pearl Double more reduction in (HFS) was observed as compared to 'Mexican Single'

particularly at T_6 and T_7 (Fig. 1). Average height of flowering stem (15.94) was calculated for Mexican Single cultivar which was higher than (14.81) taken for Pearl Double (Table 1).Significant decrease in fresh weight of flowering stem, plant height, shoot fresh and shoot dry weight was observed in cultivar 'Pearl Double' in contrast of cultivar 'Mexican Single' (Supplementary Table 2). In the same way, a significant decrease in the height of flowering stem, flowering stem diameter and fresh weight of flowering stem was noted in the means of irrigation intervals at T_6 and T_7 as compared to T_1 (Fig. 1). All these observations and findings indicate clearly that tuberose crop might be sensitive to drought stress and need frequent irrigation. A significant correlation of leaf area was seen with most of other characteristics studied including HFS, FSD, FWFS, DWFS, PH, SHFW, SHDW, LWP, LOP, LTP, RWC, A, E, WUE, SC, Chl, SOD, CAT, POD, Pro and Gly. Similarly behavior of other growth traits was observed (Fig. 2).

Leaf water relation parameters

Applying different irrigation intervals affected significantly leaf water potential (LWP), leaf osmotic potential (LOP), leaf turgor potential (LTP) and relative water content (RWC) (Supplementary Table 1). Leaf water potential was significantly influenced by different irrigation intervals and it started to decrease with increasing irrigation interval. Minimum leaf water potential value (-1.155) was recorded at T_7 (8 days irrigation interval) while the maximum leaf water potential (-0.841) was at T_1 (Fig. 1). Overall 'Mexican Single' leaves LWP was found higher (-0.941) than 'Pearl Double' (-1.076) (Table 1). Both the cultivars showed highly significant effect as 'Pearl Double' showed a decrease in leaf water potential up to treatment (T_5) consistently i.e. 6 days irrigation interval; then it showed increase in leaf water potential at T_6 in comparison with 'Mexican Single' which also showed decrease in leaf water potential up to T_5 and then indicated sudden increase at T_6(Fig. 1).Overall 'Mexican Single' leaves LOP was found to be higher in amount than 'Pearl Double' amounting as (-1.972) was recorded for 'Mexican Single' and (-2.274) for 'Pearl Double' respectively (Table 1). Leaf osmotic potential (LOP) was significantly affected by different irrigation intervals and it started to decrease with increasing irrigation interval up to T_7 (Fig. 1). Minimum leaf osmotic potential (-2.587) was recorded at T_7 (8 days irrigation interval) while the maximum leaf water potential (-1.743) was at T_1 (Fig. 1). The highest average leaf turgor potential (LTP, 0.619) was recorded in 'Mexican Single' leaves while it was amounted (0.552) in 'Pearl Double' (Table 1). Leaf turgor potential (LTP) was significantly affected by different irrigation intervals and it started to decrease with increasing irrigation interval up to T_7 (Fig. 1). Minimum leaf turgor potential (0.299) was recorded at T_7 (8 days irrigation interval) while the maximum leaf turgor potential (0.841) was at T_1 respectively (Fig. 1). Relative water content was found to be decreased as the irrigation interval increased starting from 2 days interval (T_1) to 8 days interval (T_7) in both tuberose cultivars leaves however at (T_1) and (T_2), 'Pearl Double' retained more RWC as compared to 'Mexican Single' (Fig. 1). Overall 'Mexican Single' leaves RWC was found to be higher in amount than 'Pearl Double' (Table 1). The decrease in the means of all these leaf water relation traits was noted (Fig. 1). RWC exhibited a significant correlation with all the leaf water relations, physiological and biochemical characteristics (Fig. 2)

Physiological characteristics

The application of different irrigation intervals affected significantly all physiological traits studied i.e., photosynthesis rate (A), transpiration rate (E), water use efficiency (WUE), stomatal conductance (SC) and chlorophyll contents (Chl) (Supplementary Table 1). The photosynthesis rate (A) was found to be significantly different between two cultivars subjected to different irrigation intervals; however, at T_1 (2 days intervals) no difference was observed (Table 1). Significant decreases in photosynthesis rate (A) were seen against treatment (T_3) in both cultivars; however, 'Pearl Double' showed larger & greater decrease in this regard as compared to 'Mexican Single'. The minimum amount of photosynthesis rate (2.04) was observed at longer irrigation interval (T_7) while maximum amount (8.20) at short interval (T_1). Among treatments T_4, T_5, T_6 and T_7, significant differences were noticed (Fig. 1). The influence of irrigation intervals on transpiration rate (E) was found to be differed between both cultivars and irrigation treatments and it decreased with increase of longer irrigation interval. 'Pearl Double' showed significant decrease in transpiration rate (E) after treatment T_3 (4 days interval) as compared to Mexican Single (Table 1); however, so far water interval treatments concerned, significant increase in transpiration rate (E) was noticed at T_1 and T_2 (Fig. 1). Water use efficiency of both 'Mexican Single & Pearl Double'

was significantly found to be enhanced by different irrigation intervals after first treatment (T_1) and it started to increase with increasing irrigation interval up to T_7 (Fig. 1). Maximum value of water use efficiency (7.39) was recorded at T_7 (8 days irrigation interval) and minimum value (4.10) at T_1 (Fig. 1). Both the cultivars also showed highly significant effect with each other as both started to increase water use efficiency from (T_2) i.e., 3 days interval up to treatment (T_7) i.e., 8 days irrigation interval consistently. Overall 'Pearl Double' WUE was significantly higher than 'Mexican Single' as (6.13) value was recorded for 'Pearl Double' whereas (5.07) for 'Mexican Single' (Table 1and 4). It was also revealed that longer irrigation interval caused to decrease the stomatal conductance starting from T_1 to T_7 in both the cultivars (Fig. 1). The highest average value of stomatal conductance (133.23) was recorded in 'Mexican Single' cultivar whereas (128.25) was noted in 'Pearl Double 'cultivar (Table 1). Significant increase in stomatal conductance (SC) was noticed at T_1 and T_2 (Fig. 1). Chlorophyll content (Chl) of both the cultivars was influenced significantly by different irrigation intervals; and it started to decrease with increasing irrigation interval starting from first treatment (T_1) up to T_7 (Fig. 1). Highest average value of chlorophyll content (4.44) was noted in 'Mexican Single' cultivar whereas (4.20) was noted in 'Pearl Double' cultivar (Table 1). Cultivar 'Mexican Single' contained significantly higher amounts of chlorophyll content than in 'Pearl Double' particularly at longer irrigation intervals ($T_6$and T_7); however, 'Pearl Double' retained higher chlorophyll at initial treatments like $T_1$and T_2 (Fig. 1). Maximum average value of chlorophyll content (5.49) was recorded with T_1 (2 days irrigation interval) and minimum (1.93) at T_7 (8 days irrigation interval) was obtained (Fig. 1). A significant correlation of chlorophyll content was seen with most of other traits studied including HFS, FSD,FWFS, DWFS,PH,LA, SHFW, SHDW,LWP, LOP, LTP, RWC, A, E,WUE, SC, SOD, CAT, POD, Pro and Gly (Fig. 2). A significant correlation of gas exchange traits (A, E and SC) was observed with physiological and biochemical characteristics like RWC, Chl, WUE, SOD, CAT, POD, proline and glycinebetaine contents (Fig. 2).

Table-1. The variations of the growth, leaf water relations, physiological, enzymatic and biochemical characteristics between tuberose cultivars

Traits	Cultivars		LSD (5%)
	Mexican Single	Pearl Double	
HFS (cm)	15.9±0.68a	14.81±1.02b	0.9
FSD (cm)	0.52±0.017a	0.493±0.027b	0.03
FWFS (g)	29.9±1.38a	26.42±2.38b	1.7
DWFS (g)	4.8±0.31a	4.32±0.39b	0.2
PH (cm)	23.0±0.85a	21.13±1.41b	1.3
LA (cm^2)	39.9±2.70a	37.54±3.16a	2.4
SHFW (g)	34.5±2.17a	30.92±2.98b	2.08
SHDW (g)	7.76±0.51a	5.84±0.79b	0.4
LWP (-MPa)	-0.9±0.026a	-1.076±0.039b	0.06
LOP (-MPa)	-1.9±0.057a	-2.2±0.092b	0.12
LTP (MPa)	0.6±0.034a	0.5±0.045b	0.03
RWC (%)	68.0±5.32a	61.83±6.78b	4.23
A (µmol CO_2 m^{-2} s^{-1})	5.3±0.44a	4.46±0.52b	0.32
E (mmol H_2O m^{-2} s^{-1})	2.25±0.13a	2.12±0.16a	0.13
WUE (µmol CO_2 $mmol^{-1}$ H_2O)	5.07±0.19b	6.13±0.33a	0.34
Gs (mmol m^{-2} s^{-1})	133.23±8.83a	128.25±10.1a	8.26
Chl (mgg^{-1} F. wt)	4.44±0.26a	4.20±0.37b	0.27
SOD (units / mg protein)	14.07±0.71b	16.41±0.93a	0.94
CAT (units / mg protein)	0.051±0.003b	0.065±0.00a	0.03
POD (units / mg protein)	0.284±0.020b	0.364±0.03a	0.02
Proline (µmol g^{-1}F.wt.)	4.44±0.319b	5.69±0.45a	0.32
Gly (µmol g^{-1}F.wt.)	2.26±0.103b	2.66±0.15a	0.15

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

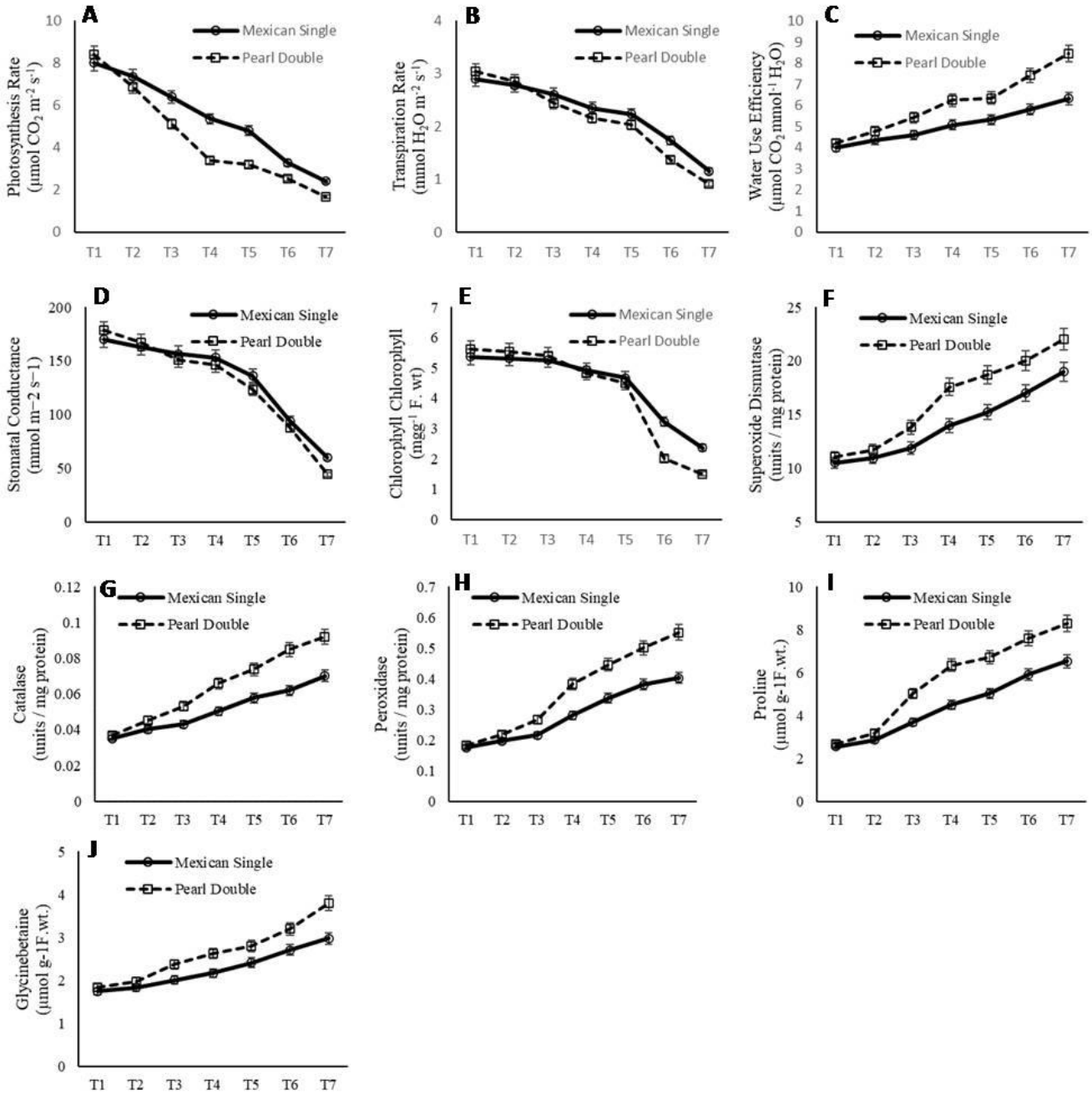

Figure-1. Effects of irrigation intervals on growth, leaf water relations, physiological, enzymatic and biochemical characteristics of tuberose cultivars.

A: Photosynthesis rate, B: Transpiration rate, C: Water use efficiency, D: Stomatal conductance, E: Chlorophyll content, F: Superoxide dismutase, G: Catalase, H: Peroxide dismutase, I: Proline content, J: Glycinebetaine content

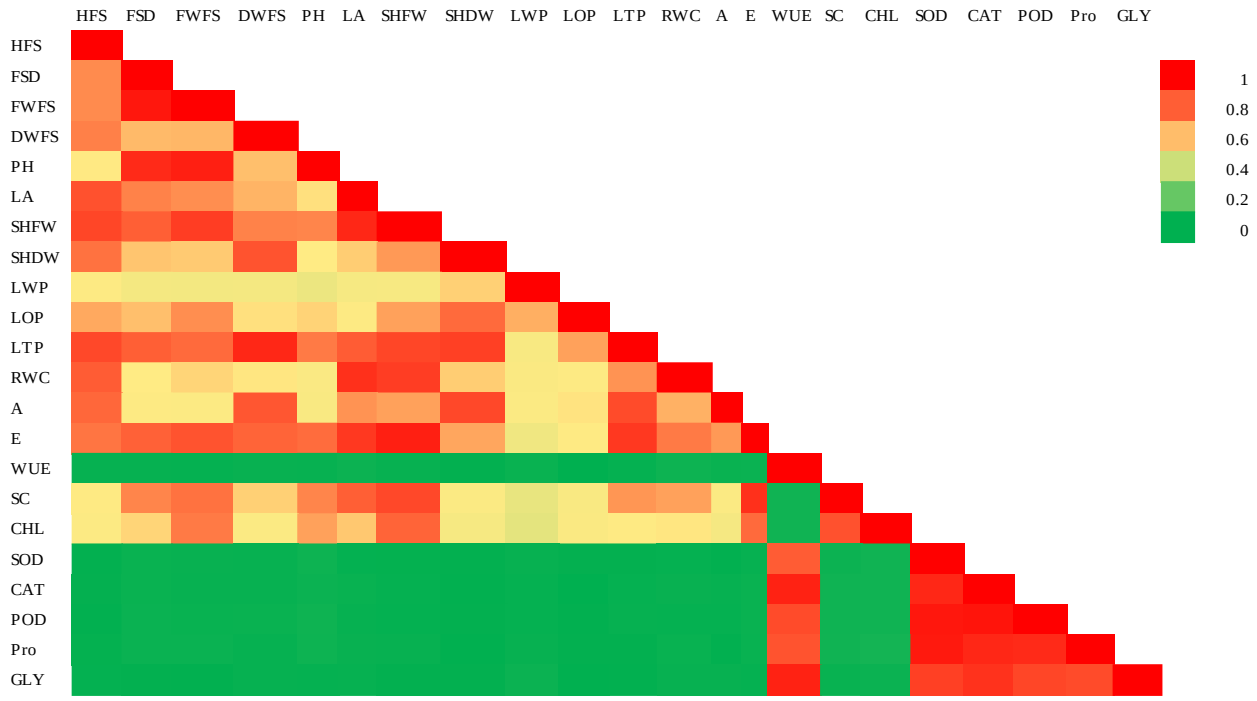

Figure-2. Correlation coefficient matrix of growth, leaf water relations, physiological, enzymatic and biochemical characteristics of tuberose cultivars

Antioxidant enzymes

The application of different irrigation intervals affected significantly all the antioxidant enzymes studied i.e., superoxide dismutase (SOD), catalase (CAT), Peroxide dismutase (POD) (Supplementary Table 1).The SOD activity increased significantly as the irrigation interval increased. The highest average SOD (16.41) was recorded in 'Pearl Double' leaves while it was (14.07) in 'Mexican Single (Table 1). SOD was significantly affected by different irrigation intervals and it started to increase with increasing irrigation interval up to T_7 (Fig. 1). Minimum SOD (10.76) was recorded at T_1 (2 days irrigation interval) while the maximum SOD activity (20.50) at T_7; however, there was seen no significant difference among $T_1$and T_2 (Fig. 1).

The CAT activity increased significantly as the irrigation interval increased. The highest average CAT (0.065) was recorded in 'Pearl Double' leaves while it was (0.051) in 'Mexican Single' however there was found no significant difference between both cultivars at T_1 (2days interval). CAT was significantly affected by different irrigation intervals and it started to increase with increasing irrigation interval up to T_7 (Fig. 1). Minimum activity (0.036) was recorded at T_1 (2 days irrigation interval) while the maximum CAT activity (0.081) at T_7 respectively (Fig. 1).

The POD activity increased significantly as the irrigation interval increased (Fig. 1). The highest average POD (0.364) was recorded from 'Pearl Double' leave samples while it was (0.284) in 'Mexican Single' however there was found no significant difference between both cultivars at T_4(5day interval) and T_7(7 days interval) (Table 1). POD was significantly affected by different irrigation intervals and it started to increase with increasing irrigation interval up to T_7 (Fig. 1). Minimum activity (0.179) was recorded at T_1 (2 days irrigation interval) while the maximum POD activity (0.476) at T_7 respectively (Fig. 1).

Biochemical parameters

The proline content (Pro) activity increased significantly as the irrigation interval increased. The highest average Proline activity (5.695) was recorded from 'Pearl Double' leaves while it was (4.44) in 'Mexican Single' however there was found no significant difference between both cultivars at T_7 (7day interval) (Table 1). Proline was significantly affected by different irrigation intervals and it started to increase with increasing irrigation interval up to T_7 (Fig. 1). Minimum proline content (2.63) was recorded at T_1 (2 days irrigation interval) while the

maximum (7.41) at T_7 (longer irrigation interval) (Fig. 1). Proline exhibited significant correlation with almost all parameters studied such as HFS, FSD, FWFS,DWFS, PH, LA, SHFW, SHDW, LWP, LOP, LTP, RWC, A, E, WUE,SC, Chl, SOD, CAT,POD and Gly (Fig. 2).The glycinebetaine content (Gly) increased significantly as the irrigation interval increased. The highest average glycinebetaine activity (2.66) was recorded in cultivar 'Pearl Double' leaves while it was (2.26) in cultivar 'Mexican Single' however there was found no significant difference between both cultivars at T_1 (2day interval) at the start (Table 1). Glycinebetaine was significantly affected by different irrigation intervals and it started to increase with increasing irrigation interval up to T_7 (Fig. 1). Minimum glycinebetaine content (1.79) was recorded at T_1 (2 days irrigation interval) while the maximum (3.38) at T_7 (longer irrigation interval), respectively (Fig. 1).

Discussion

Effect of irrigation intervals on vegetative growth characters

In current studies, water deficit caused a remarked reduction in all these growth characters. Water deficit is characterized by decreased water content in the leaves and consequently leaf water potential becomes reduced, closing of stomata occurs, and growth is decreased at the end. Water deficit stress severity can lead to photosynthesis binding, metabolism disruption, and eventually plant death (Jaleel et al., 2008). Prolonged irrigation has reduced the vegetative and reproductive growth of both the tuberose cultivars (Supplementary Table 2). In 'Pearl Double' cultivar, with increase of drought stress, height of flowering stem decreased more drastically as compared to 'Mexican Single' cultivar (Supplementary Table 2and 3). These results indicated that both tuberose cultivars were sensitive to water deficit. Therefore, when irrigated, frequent irrigation should be used. These results are similar to those of Sepaskhah and Yarami (2009) whom concluded that water pressure obtained by lowering the water level per application reduced the production of flower of saffron and also in agreement with Moftah and Al-Humaid (2006) whom concluded that all factors like spike inflorescences in tuberose are highly reduced if tuberose plants will be grown under water stress conditions.

The decrease in flowering stem diameter, fresh and dry weight of flowering stem in the 'Pearl Double' cultivar was higher than in the 'Mexican Single' cultivar (Supplementary Table 2). Increased irrigation time significantly reduced the diameter, fresh and dry weight of the flowering stem as compared to control, besides T_6 and T_7 had a similar effect on fresh weight of flowering stem (Supplementary Table 2). Jaimez et al., (2000) reported similar results in which they revealed that dehydration significantly influenced the flowers abortion, bulb size, length of flowering spike, and variations in number of flowers per plant and also in agreement with Bahadoran and Salehi (2015) who found that tuberose cannot tolerate water and salt stress and different vegetative characteristics were negatively influenced upon occurrence of these stresses on two tuberose cultivars e.g. ('Mahallati' and 'Dezfuli'). The initial reaction of almost all plants to severe dehydration is that their stomata are closed to stop water loss which occurs through the process of transpiration. Closing of stomata due to dehydration pressure in particular will lead to a decrease in photosynthesis (Mahajan and Tuteja, 2005). Decreased photosynthesis and reduced transport of photosynthesis products to flowering spikes will decrease flower formation and yield and ultimately decrease in reproductive variables. Moreover, it causes reduction in plant height as apparent from our finding where minimum plant height was noted at higher irrigation interval (stress) i.e. 8 days interval respectively (Fig. 1). The similar decrease in height of plant was also observed by Ram et al., (1999) in winter flower marigold, Nagaraju et al., (2003) in rose cultivars and Halepyati et al., (2002) in tuberose due to water deficit. Same behavior was noticed in sunflower.

These results indicated that growth characteristics like leaf area and some other morphological attributes such as shoot dry weight of tuberose reduced mainly under water deficiency treatments, especially at T_6 and T_7 (7 and 8 days irrigation interval) as compared with the control (T_1) (Fig. 1). These outcomes are also in agreement with Younis et al., (2000) and Taiz and Zeiger (2002) who stated that the water stress in plants stops the leaf expansion, stem elongation and root growth and development. Similar findings were obtained by Chawla (2008) in African marigold. Hence, a minor decrease in water status, water potentials and turgor potential can either slow down or completely cease plant growth.

Data showed that shoot fresh and dry weight of tuberose were significantly reduced by water deficit treatments, particularly at T_6 and T_7 (7 and 8 days

interval regimes) in comparison to the control (T_1) (Supplementary Table 2 and 4). Furthermore, this drop in dry weight of shoots (7.76) in 'Mexican Single' cultivar was observed comparatively more than in 'Pearl Double' which showed average decrease (5.84) (Table 1). Results of this study also in consistent with many other previous studies; Moftah and Al-Humaid (2006) observed decrease in shoot fresh and dry biomass of tuberose plants under irrigation stress; Navarro et al., (2007) described about major reduction in the biomass of plant tissues of *Arbutus unedo* L. in drought stress and the results of Fornes et al., (2007) in ornamental plants subjected to water shortage, Shillo et al. (2002) in cut flower and some bulb species. Egert and Tevini (2002) also reported remarkable decrease n in the dry matter and chlorophyll content of in the leaves of Chives and Bass et al., (1995) in Carnation and Gerbera under drought stress.

It is resulted that increase in irrigation levels significantly reduced many vegetative attributes. A reduction in plant height at higher irrigation intervals may be due to less water availability to plant. Moisture deficit resulted in the decrease in carbohydrates translocation, growth hormones and nitrogen metabolisms disturbance which led to the further pressure loss and as a result inhibited the growth (Verasan and Phillips, 1978). Due to frequent irrigation, plants can maintain higher water potential. Decreasing irrigation interval can improve and affect the physiological and biochemical activities in better way. Cell division and cell enlargement are the main factors for growth and require ideal supply of water (Slatyer, 1970). All these reports clearly emphasized that the availability of water is pre requisite for better metabolism and speedy growth and development in all the plants.

EL-Naggar and Byari (2009) also concluded that irrigating tuberose plants after every two days significantly increased total bulb yield, expressed as number per clump. This treatment produced the highest yield of small bulblets or cloves, weight and size per clump, to other watering frequency treatments.

Effect of irrigation intervals on leaf water relation attributes

The relative water content (RWC) of leaves reduced in all water deficit treatments as watering time increased (Fig. 1); e.g., Minimum water content (26.08%)with T_7 (8 days of irrigation) was recorded and maximum at T_1 (95.48 %) respectively (Fig. 1). Similarly, Anyia and Herzog (2004) stated that an important correlation between CO_2 and RWC regulation was confirmed for maintaining balance in water content and stomatal conductance of cowpea (*Vigna unguiculata* L.) in drought conditions in their study.

In addition, the reduction in water related attributes in drought stressed plants has also been documented by De Pascale et al. (2003). It is described that when water absorption occurs less than respiration in plants, then turgor pressure of plant tissues becomes down and in result of this cell RWC reduced, whereas concentration in cellular solutes increases, ultimately the leaf osmotic potential ($\Psi\pi$) and leaf water potential (Ψw) both fall down (Lawlor and Cornic, 2002; Jifon and Syvertsen, 2003). Less turgor and RWC slow down the plant growth and development consequently and hence reduced stomatal conductance (gs).

Effect of irrigation intervals on physiological attributes

Plant water conditions, photosynthetic (A) and respiratory (E) rates were significantly lower during periods of high irrigation at T_6 and T_7 (water-stressed tuberose plants) relative to $T_1$and T_2 (stress free plants) at all stages of growth (Fig. 1). The data showed that a decrease in photosynthetic levels (A), net CO_2 intake and transpiration rate (E) were associated with a significant decrease in stomatal conduction (gs). There have been proposed three types of evidences which point out about the remarkable influence of low RWC on photosynthetic levels (A). The first one is in agreement with Lawlor and Cornic (2002), who stated that decrease in RWC causes to reduce stomatal conductance (gs) as well as the accumulation of CO_2 (Ci) inside the leaf. As a result, total amount of CO_2 (A) estimate decreases according to the equation: A = gs [Ci]. A second set of evidence suggests that CO_2 exposure to the photosynthetic enzyme "Rubisco" can become limited due to physical changes in intercellular spaces formation because of leaf shrinking at low RWC (Lawlor and Cornic, 2002). Last third clarification was described by Prakash and Ramachandran (2000) who argued that photosynthetic levels 'decline was mainly because of reduction in chlorophyll content in serious dehydration circumstances.

In consonance with present research work and previous reports by Liang et al., (2002); stomatal conduction (g*s*) and transpiration rate (E) were

significantly decreased when leaf water potential (Ψw) and RWC decrease under water deficit conditions. The influence of lower stomatal conductance (gs) on reducing transpiration rate by decreasing water availability may be a combination of a few events. Some of these conditions can include enhanced hydraulic resistance between the xylem cells, increased hindrance to the interphase of soil roots (Passioura, 1988), and an increased irradiation energy falling on surface of leaves (Taiz and Zeiger, 2002).

Increasing exposure to concentrated radiations on the surface of tuberose leaves may be the cause of the large increase in WUE exposure to water-suppressed plants at large water intervals. In conclusion, detrimental effects of deficiency of water content during photosynthesis, growth and transpiration phenomenon in tuberose crop, which is an important bulb plant, is the main factor to be kept in consideration in arid areas. Under high water deficiency conditions in both arid and semi-arid regions, frequent irrigation (short interval irrigation) can reduce leaf temperature and ultimately transpiration rate; as well as enhance photosynthetic rate and consequently water use efficiency (WUE). Similarly, EL-Naggar and Byari (2009) reported that irrigation after every two and / or four days greatly increased the cut flower yield of tuberose, increased flowering time and significantly increased diameters and circumferences, compared with regular irrigation in six or eight days. Irrigation after every four and / or six days dramatically increased the yield of tuberose flower stem, water use efficiency (WUE) as unit fresh weight.

Increasing drought levels reduced the chlorophyll content (Chl) in the leaves of both cultivars as compared to controls. There was a slight reduction in chlorophyll content from T_1 to T_5, in the plants of both cultivars, however, this decrease was enhanced at T_6 to T_7 as compared to controls (Fig. 1). Mild water stress increased total chlorophyll (Chl) and did not become the cause of major reduction of chlorophyll content. Chl a and Chl b both might contribute to increase the process. However, as water deficiency became heavier, all types of chlorophyll content were decreased during plant growth phases compared to control. Present findings were in agreement with those presented by Younis et al., (2000), who concluded that low and short-term water deficiency enhanced all above three types of chlorophyll contents in various sorghum products, while long term drought reduced chlorophyll remarkably. By comparing the means, 'Mexican Single' had average (4.44) chlorophyll content which was slightly more than 'Pearl Double' (4.20) (Table 1). Reduced amount of chlorophyll content (1.93) at the treatment T_7 (8 days interval) was observed sequentially. This assessment of reduced chlorophyll content may be because of the enhanced leaf stiffness and due to the closeness of compressed mesophyll cells in water stressed leaves, as a result, production of too many chloroplasts in limited cellular area, as is mostly observed in water deficit conditions (Delperee et al., 2003). Similar effects of decreasing chlorophyll in brinjal in water deficit were described by Prakash and Ramachandran (2000) who hypothesized that Chl a precursor, actually reduces the total chlorophyll content under moisture deficiency and it happens due to inhibited biosynthesis of this precursor. Similarly, reduction of chlorophyll content in *Hordeum vulgare* L. was also reported by Mamnouie et al., (2006) under drought circumstances. Prolonged irrigation can enhance and create oxidative pressure in drought stress; and might decreased chlorophyll in *H. vulgare* and it might be due to the fact that plants adapt to such drought conditions under oxidative stress. Similarly, oxidative stress condition might be the cause of decreasing chlorophyll in the leaves of tuberose plant (Seel et al., 1992).

Effect of irrigation intervals on enzymatic and biochemical attributes

Activity of (SOD), catalase (CAT) and peroxide dismutase (POD) was higher during water deficit condition and it was observed less in control plants during shorter irrigation intervals. Maximum enzyme activity was recorded with T_7 (8 days irrigation interval) and minimum atT_1 (2 day interval). Enzyme activity in tuberose increased as the stress level became higher in both cultivars. These findings were in consistent agreement with previous studies conducted by Li and Feng (2011), Shahana et al., (2015). The elevation of SOD, CAT, and POD enzyme activities during increased drought stress is a plant's adaptive response to protect itself from oxidative damage caused by the accumulation of reactive oxygen species. These enzymes play a crucial role in maintaining cellular homeostasis and preventing cellular damage, ultimately enhancing the plant's survival under drought conditions (Mahesh et al., 2013).

The proline and Glycinebetaine content were observed to be increased in both cultivars 'leaves at

longer irrigation interval. Irrigation intervals for T_6 and T_7, showed significant increases in proline (Fig. 1). The 'Pearl Double' cultivar showed higher and significant increase in proline (while non-significant for Glycinebetaine) content than the 'Mexican Single' cultivar (Table 1). Mahajan and Tuteja (2005) stated that to preserve the water in cell as well as cellular proteins, during water stress, plants store different metabolites called "compatible solutes." These solutions do not prevent regular metabolic reaction processes. Commonly seen metabolic products which have the function of osmolytes were especially sugars, sucrose and fructose and different other complex sugars such as fructans and trehalose. In addition, proline and glycinebetaine also assembled and their concentration helps to form osmotic adjustment gradient and the concentration of these osmolytes reduces water intake within the cell and stops the loss of water molecules inside the cell (Mahajan and Tuteja, 2005). Increase of proline content due towater stress was similar to the findings of Jampeetong and Brix (2009) who studied and revealed an enhancement in proline content and a reduction in chlorophyll content of *Salvinia natans* L. in stressful conditions.

Conclusion

It is concluded that longer irrigation interval viz. T_6 (7 days) and T_7 (8 days) could damage tuberose crop at cellular level; however tuberose cultivars were independent of water deficit injuries. Increased photosynthetic and stomatal conductance rates at higher and longer intervals suggests that 'Mexican Single' is more capable of absorbing, retaining water and mobilizing to its leaves and consequently synthesizing more food and nutrients for crop better growth and development. Collectively, these results suggest that cultivar 'Mexican Single' has more tolerant to moisture stress than cultivar 'Pearl Double'.

Contribution of Authors

Ali S: Conceived idea, designed research methodology, performed experiment, collected and analysed data and manuscript write up
Balal RM: Supervised the study, edited and approved final draft of manuscript
Javaid MM: Literature review, data analysis & interpretation and manuscript write up

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Supplementary Table 1. Analysis of variance for growth, leaf water relations, physiological, enzymatic and biochemical traits of tuberose cultivars.

S.V	Irrigation	Variety	Irrigation x V	Error	CV (%)
D.F	6	1	6	28	
HFS	89.9**	13.4*	4.9^{NS}	2.2	9.83
FSD	0.05**	0.01**	0.00^{NS}	0	9.71
FWFS	454.3**	132.2**	38.8**	7.9	8.98
DWFS	16.08**	3.48**	0.39^{NS}	0.216	10.08
PH	154.5**	37.6**	13.4*	4.6	9.79
LA	1118.9**	61.7 ^{NS}	16.7^{NS}	15.1	10.06
SHFW	873.3**	134.4**	27.4*	10.9	10.1
SHDW	55.5**	38.8**	3.5**	0.5	10.4
LWP	0.19**	0.19**	0.00^{NS}	0	8.64
LOP	0.57**	0.95**	0.04^{NS}	0.04	9.67
LTP	0.20**	0.04**	0.00^{NS}	0	9.98
RWC	4894.4**	399.9**	93.7^{NS}	45	10.34
WUE	7.92**	11.73**	0.67^{NS}	0.29	8.78
SC	11754.7**	260.0^{NS}	112.9^{NS}	171.4	10.01
CHL	12.63**	0.61^{NS}	0.49*	0.18	10.02
SOD	82.7**	57.4**	2.4^{NS}	2.2	9.82
CAT	0.00**	0.00**	0.00*	0	9.96
POD	0.08**	0.06**	0.00**	0	10.17
Pro	19.67**	16.38**	0.78*	0.26	10.15
GLY	1.95**	1.62**	0.08^{NS}	0.05	9.81

* = Significant (P<0.05); ** = Highly significant (P<0.01); NS = Non-significant (P>0.05);
HFS: Height of flowering stem, FSD: Flowering stem diameter, FWFS: Fresh weight of flowering stem, DWFS: Dry weight of flowering stem, PH: plant height, LA: Leaf area, SHFW: Shoot fresh weight, SHDW: Shoot dry weight:LWP: Leaf water potential, LOP: Leaf osmotic potential, LTP: Leaf turgor potential, RWC: Relative water content, A: Photosynthesis rate, E: Transpiration rate, WUE: Water use efficiency, SC: Stomatal conductance, CHL: Chlorophyll content, SOD: Superoxide dismutase, CAT: Catalase, POD: Peroxide dismutase, Pro: Proline content, GLY: Glycinebetaine content

Supplementary Table 2. Effect of irrigation intervals on growth parameters of two tuberose cultivars

Treatment		T_1	T_2	T_3	T_4	T_5	T_6	T_7	Mean		SEM±
Height of flowering stem (cm)	Single	20.0± 1.1	18.6± 1.0	18.0± 0.9	15.6±0.8	14.0±0.7	13.4±0.7	12.0±0.6	15.9±0.6A	I	0.8724
	Double	21.2± 1.1	19.2± 1.0	17.7± 0.9	14.2±0.7	12.6±0.7	10.7±0.5	8.0±0.4	14.8±1.02B	V	0.4663
	Mean	20.6± 0.7A	18.9± 0.6A	17.8± 0.6A	14.9±0.6B	13.3±0.5BC	12.0±0.7CD	10.0±0.9D	-	IxV	1.2337
Flowering stem diameter (cm)	Single	0.6± 0.03	0.5± 0.03	0.5± 0.03	0.5±0.02	0.5±0.02	0.4±0.02	0.4±0.02	0.5±0.01A	I	0.0286
	Double	0.630± 0.03	0.601± 0.03	0.540± 0.02	0.505±0.02	0.484±0.02	0.427±0.02	0.266±0.01	0.493± 0.02B	V	0.0153
	Mean	0.61± 0.02A	0.59± 0.02AB	0.55± 0.02ABC	0.52±0.01BCD	0.49± 0.01CD	0.45±0.02D	0.33±0.03E	-	IxV	0.0404
Fresh weight of flowering stem (g)	Single	36.6± 2.02ab	34.8± 1.92ad	33.7± 1.86ad	31.1±1.72ae	29.3±1.62be	24.9±1.37efg	19.0±1.05fg	29.9±1.38A	I	1.6247
	Double	38.5± 2.12a	35.5± 1.96abc	32.5± 1.79ae	28.1±1.55cde	26.5± 1.46def	17.5±0.97g	6.1±0.34h	26.4±2.38B	V	0.8684
	Mean	37.5± 1.37A	35.1± 1.24A	33.1± 1.19AB	29.6±1.24BC	27.9±1.16 C	21.2±1.81 D	12.6±2.93 E	-	IxV	2.2977
Dry weight of flowering stem (g)	Single	7.0± 0.39	5.7± 0.32	5.4±0.30	5.0±0.28	4.6±0.26	3.8±0.21	2.5±0.14	4.8±0.31A	I	0.2682
	Double	7.4± 0.4	5.6± 0.3	4.5±0.2	4.1±0.2	4.0±0.2	2.5±0.1	1.8±0.1	4.3±0.3B	V	0.1433
	Mean	7.2± 0.2A	5.6± 0.2B	5.0±0.2BC	4.5±0.2C	4.3±0.2C	3.2±0.2D	2.1±0.1E		IxV	0.3793
Plant height (cm)	Single	27.0± 1.49a	25.9± 1.43ab	24.3± 1.34ab	23.7±1.31ab	23.4±1.30ab	20.2±1.12bcd	16.4±0.91cd	23.0±0.85A	I	1.2481
	Double	28.3±	26.5±	22.8±	22.2±1.2a-d	22.6±1.2a-d	16.3±0.90d	9.0±0.50e	21.13±	V	0.6671

		1.5a	1.4ab	1.2abc					1.41B		
	Mean	27.6± 1.0A	26.2± 0.9AB	23.5± 0.8B	22.9±0.8B	23.0±0.8B	18.2±1.0C	12.74±1.7D	-	IxV	1.7651
Leaf area (cm^2)	Single	52.00± 2.8	50.96± 2.8	50.44± 2.7	43.16±2.38	34.32±1.8	29.12±1.6	19.76±1.0	39.97±2.7A	I	2.2502
	Double	54.6± 3.0	52.9± 2.9	46.4± 2.5	37.8±2.0	30.5±1.6	26.2±1.4	14.2±0.7	37.5±3.1A	V	1.2028
	Mean	53.3± 1.9 A	51.9± 1.8 A	48.4± 1.9 A	40.5±1.8B	32.4±1.4C	27.6±1.1C	16.9±1.3D	-	IxV	3.1823
Shoot fresh weight (g)	Single	45.00± 2.48ab	42.75± 2.36ab	41.40± 2.28abc	37.35±2.06bc	32.00± 1.76cd	25.00± 1.38def	18.00± 0.99efg	34.50± 2.17A	I	1.9115
	Double	47.25± 2.61a	43.59± 2.40ab	39.88± 2.20abc	32.79±1.81cd	27.00±1.49de	16.73±0.92fg	9.21±0.51g	30.92± 2.98B	V	1.0217
	Mean	46.13± 1.69 A	43.17± 1.52 A	40.64± 1.46 AB	35.07± 1.59 BC	29.50± 1.52 C	20.86± 1.99 D	13.61±2.03 E	-	IxV	2.7033
Shoot dry weight (g)	Single	11.00± 0.61a	9.90± 0.55ab	8.47± 0.47bc	7.81±0.43bcd	7.26±0.40cde	5.94±0.33def	3.96±0.22fg	7.76±0.51A	I	0.4112
	Double	11.55± 0.64a	9.76± 0.54ab	6.37± 0.35cde	5.52±0.30ef	4.00±0.22fg	2.52±0.14gh	1.16±0.06h	5.84±0.79B	V	0.2198
	Mean	11.28± 0.41 A	9.83± 0.34 B	7.42± 0.54 C	6.67±0.56 CD	5.63±0.76 D	4.23±0.78 E	2.56±0.64 F	-	IxV	0.5816

Means sharing similar letter in a row or in a column are statistically non-significant (P>0.05). Small letters represent comparison among interaction means and capital letters are used for overall mean.

Supplementary Table 4. Effects of irrigation intervals on growth, leaf water relations, physiological, enzymatic and biochemical characteristics of tuberose cultivars

	Treatments						
Traits	T_1	T_2	T_3	T_4	T_5	T_6	T_7
HFS	20.60±0.77A	18.90±0.67A	17.85±0.63A	14.90±0.61B	13.31±0.56BC	12.05±0.74CD	10.03±0.95D
FSD	0.615±0.022A	0.591±0.021AB	0.555±0.020ABC	0.520±0.019BCD	0.497±0.018CD	0.456±0.021D	0.334±0.033E
FWFS	37.59±1.37A	35.18±1.24A	33.12±1.19AB	29.64±1.24BC	27.95±1.16C	21.25±1.81D	12.61±2.93E
DWFS	7.23±0.26A	5.69±0.20B	5.01±0.26BC	4.59±0.24C	4.33±0.21C	3.20±0.29D	2.19±0.17E
PH	27.68±1.01A	26.26±0.93AB	23.56±0.89B	22.99±0.87B	23.06±0.83B	18.28±1.09C	12.74±1.73D
LA	53.30±1.95A	51.93±1.86A	48.43±1.92A	40.53±1.84B	32.45±1.41C	27.66±1.16C	16.98±1.38D
SHFW	46.13±1.69A	43.17±1.52A	40.64±1.46AB	35.07±1.59BC	29.50±1.52C	20.86±1.99D	13.61±2.03E
SHDW	11.28±0.41A	9.83±0.34B	7.42±0.54C	6.67±0.56CD	5.63±0.76D	4.23±0.78E	2.56±0.64F
LWP	-0.841±0.031A	-0.873±0.033A	-0.928±0.040AB	-1.018±0.049ABC	-1.153±0.059C	-1.092±0.056BC	-1.155±0.065C
LOP	-1.743±0.064A	-1.832±0.070A	-1.950±0.085AB	-2.111±0.102ABC	-2.212±0.104BCD	-2.429±0.136CD	-2.587±0.159D
LTP	0.841±0.031A	0.754±0.026A	0.641±0.031B	0.592±0.033B	0.537±0.021BC	0.434±0.029C	0.299±0.033D
RWC	95.48±3.49A	93.03±3.34A	84.12±3.02AB	73.94±3.22B	46.11±3.52C	35.63±3.08CD	26.08±3.06D
A	8.20±0.30A	7.13±0.27B	5.76±0.35C	4.39±0.46D	4.00±0.39D	2.90±0.20E	2.04±0.18E
E	2.97±0.11A	2.82±0.10AB	2.53±0.10BC	2.26±0.09CD	2.13±0.09D	1.56±0.10E	1.04±0.07F
WUE	4.10±0.15D	4.57±0.19D	5.01±0.26CD	5.66±0.33BC	5.83±0.31BC	6.61±0.43AB	7.39±0.55A
Gs	174.25±6.37A	165.32±5.84AB	153.53±5.51ABC	149.69±5.43BC	129.58±5.36C	90.73±3.49D	52.09±3.99E
Chl	5.49±0.20A	5.42±0.20A	5.33±0.19AB	4.87±0.17AB	4.58±0.16B	2.61±0.29C	1.93±0.20C
SOD	10.76±0.39C	11.31±0.43C	12.84±0.63C	15.77±0.98B	16.97±0.98B	18.50±0.93AB	20.50±0.98A
CAT	0.036±0.001F	0.043±0.002EF	0.048±0.003DE	0.058±0.004CD	0.066±0.004BC	0.073± 0.006AB	0.081±0.006A
POD	0.179±0.007E	0.207±0.009DE	0.241±0.014D	0.331±0.026C	0.391±0.028BC	0.440± 0.031AB	0.476±0.037A

Pro	2.634±0.096E	3.039±0.128E	4.357±0.331D	5.419±0.455C	5.876±0.428BC	6.756± 0.446AB	7.414±0.474A
Gly	1.794±0.066D	1.909±0.074D	2.194±0.112CD	2.401±0.133C	2.608±0.125BC	2.956± 0.150AB	3.388±0.220A

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

Supplementary Table 5. Correlation coefficient matrix of growth, leaf water relations, physiological, enzymatic and biochemical characteristics of tuberose cultivars

	HFS	FSD	FWFS	DWFS	PH	LA	SHFW	SHDW	LWP	LOP	LTP	RWC	A	E	WUE	SC	Chl	SOD	CAT	POD	Pro	Gly
HFS	1.000																					
FSD	0.950	1.000																				
FWFS	0.950	0.992	1.000																			
DWFS	0.954	0.933	0.934	1.000																		
PH	0.916	0.985	0.989	0.931	1.000																	
LA	0.971	0.953	0.949	0.935	0.919	1.000																
SHFW	0.975	0.966	0.978	0.953	0.952	0.986	1.000															
SHDW	0.959	0.929	0.927	0.970	0.915	0.926	0.945	1.000														
LWP	0.907	0.835	0.832	0.839	0.778	0.853	0.857	0.925	1.000													
LOP	0.939	0.931	0.949	0.919	0.924	0.904	0.942	0.962	0.937	1.000												
LTP	0.974	0.966	0.962	0.986	0.956	0.967	0.975	0.977	0.870	0.942	1.000											
RWC	0.967	0.915	0.923	0.917	0.881	0.983	0.978	0.926	0.878	0.905	0.947	1.000										
A	0.963	0.901	0.897	0.969	0.877	0.947	0.942	0.974	0.891	0.918	0.973	0.936	1.000									
E	0.958	0.965	0.970	0.964	0.961	0.980	0.989	0.940	0.808	0.914	0.980	0.956	0.945	1.000								
WUE	-0.945	-0.946	-0.957	-0.933	-0.939	-0.907	-0.942	-0.968	-0.927	-0.997	-0.954	-0.896	-0.926	-0.922	1.000							
SC	0.914	0.952	0.959	0.925	0.952	0.966	0.974	0.887	0.746	0.871	0.946	0.942	0.890	0.983	-0.877	1.000						
CHL	0.894	0.923	0.956	0.887	0.942	0.928	0.964	0.854	0.718	0.878	0.908	0.917	0.843	0.962	-0.876	0.971	1.000					
SOD	-0.972	-0.929	-0.933	-0.942	-0.899	-0.960	-0.964	-0.974	-0.940	-0.967	-0.965	-0.954	-0.976	-0.948	0.967	-0.900	-0.877	1.000				
CAT	-0.966	-0.928	-0.940	-0.943	-0.908	-0.930	-0.956	-0.980	-0.956	-0.991	-0.959	-0.937	-0.952	-0.929	0.988	-0.880	-0.873	0.986	1.000			
POD	-0.976	-0.921	-0.932	-0.928	-0.890	-0.945	-0.960	-0.968	-0.958	-0.978	-0.952	-0.956	-0.957	-0.929	0.973	-0.881	-0.874	0.992	0.993	1.000		
Pro	-0.964	-0.915	-0.917	-0.949	-0.894	-0.933	-0.943	-0.988	-0.943	-0.969	-0.968	-0.932	-0.981	-0.932	0.970	-0.872	-0.847	0.991	0.986	0.985	1.000	
GLY	-0.960	-0.970	-0.976	-0.948	-0.959	-0.952	-0.972	-0.970	-0.912	-0.986	-0.977	-0.939	-0.944	-0.959	0.988	-0.931	-0.913	0.977	0.982	0.975	0.973	1.000

**. Correlation is significant at the 0.01 level (2-tailed); *. Correlation is significant at the 0.05 level (2-tailed)