

DROUGHT TOLERANCE OF *Leucophyllum frutescens*: PHYSIOLOGICAL AND MORPHOLOGICAL STUDIES REVEAL THE POTENTIAL XEROPHYTE

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ABSTRACT

Xeriscaping focus on the use of drought tolerant species of plants for environmental sustainability leading to the conservation of natural resources. We need to look for drought tolerant, water efficient plant species. Present research was aimed to assess the water use efficiency of *Leucophyllum frutescens* (silvery) for its adaptability and potential as xerophyte. In this study, seven treatments were applied were 100% field capacity was considered as control while 85%, 70%, 55%, 40%, 25% and 5% field capacities were taken as different drought levels. Effect of drought commenced at 40% field capacity and lower drought treatments as decrease in shoot and root fresh and dry weight, root length, leaf area and leaf area index and leaf firing percentage increased with severity of drought compared to 100% field capacity. Physiological parameters including water use efficiency (A/E), leaf water potential (ψ_{leaf}), stomatal conductance (g_s), photosynthesis rate, cell membrane stability and total chlorophyll contents proved that this plant species can tolerate severe drought conditions. Positive correlation was found among most of the attributes but leaf temperature was negatively correlated with leaf water potential, photosynthesis rate, cell membrane stability, and chlorophyll contents. Moreover, regression analysis between various morphological and physiological attributes showed the predictive power of the model yielding significant results for leaf area and cell membrane stability ($R^2 = 0.74$), root length and photosynthesis rate ($R^2 = 0.65$), leaf temperature and chlorophyll contents ($R^2 = 0.43$) and leaf area and leaf water potential (ψ_{leaf}) ($R^2 = 0.93$).

Key words: cell membrane stability; field capacity; leaf area index; *Leucophyllum frutescens*, photosynthesis rate; stomatal conductance; water potential

INTRODUCTION

Leucophyllum frutescens locally known as silvery is one of the perennial bedding plant belongs to family Scrophulariaceae. It is native to Texas, USA and grows

up to 1–2 m in height with a spread of about 0.5–1 m [Hilliard 1983]. Silvery is attractive ornamental plant with its compact shape and grey foliage can be used as

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ornamental plant and provide positive physical surroundings [Younis et al. 2002, Khan et al. 2005]. It requires less water than other annual and perennial plants and is quite tolerant of summer heat but it does not tolerate frost. In urban landscape, this plant species can be used for rock gardening, as ground covers, border plant and on slopes; it can spread rapidly and flowers mostly in early and late summer. Multipurpose usages of silvery make it important to use this plant in landscape and xeriscape. With the extensive awareness of water conservation and environment friendly practices, drought tolerant plants usage has been increased worldwide [Nadeem et al. 2012].

Artificial irrigation is becoming crucial for the suburban and urban landscape of tropical and subtropical areas, although availability of fresh water is becoming limiting factor. On the other hand, use of municipal supply water becomes scarce for household purpose as most of its proportion is used for irrigation of landscape premises due to less rain fall and high temperature during the summer season. It is assessed that every year large amount of irrigation water is being wasted and that may have pesticides, fertilizers and other hazardous chemicals that are used in maintenance of landscape [Riaz et al. 2013].

For efficient use of irrigation water, it is recommended to select only those plant species that are suitable for any designated landscape area [Reynolds 2006]. The demand for the ornamental plants with minimum water requirement is very high, particularly in dry climate regions. The sustainability of landscape in dry hot areas is fully dependent on judicious plant selection [Younis et al. 2010]. To our knowledge, the significant screening of bedding plants took place, but screening on landscape plants like *L. frutescens* was not conducted. Therefore, research is desired regarding performance evaluation of this plant species under drought stress to estimate its tolerance level therefore able to recommend it under optimum landscape settings. On the other hand, plant materials, soil and growing condition have changed greatly over the time intervening years justifying new look at tolerance in commonly grown flowering plants to combat with drought problem. In this work, we characterized the growth and physiological response of *L. frutescens* exposed to different levels of

water stress to test the hypothesis that this species can be used in drought tolerant landscape.

MATERIALS AND METHODS

The experiment was conducted during 2010–2011 at floriculture research area, Institute of Horticultural Sciences, University of Agriculture, Faisalabad, Pakistan, latitude 31°26' N, longitude 73°06' E, altitude 214 m. The climate of Faisalabad conscripted as semi-arid (BWh) in Köppen Geiger classification system having very hot humid summer and dry cool winter. The average maximum temperature during study period was in the month of June (40.5°C), while, the average minimum temperature was in October (17.5°C).

A pot experiment in the open lit green house was conducted to evaluate the effect of drought stress on the growth and development of *L. frutescens*. Seven drought levels were maintained viz. control with 100 percent field capacity, 85, 70, 55, 40, 25 and 5% field capacity during the month of April to October.

Healthy seedlings of one year were transplanted in pots (24 cm diameter and 28 cm depth) containing ~1.25 kg mixture of sand, silt and leaf compost (1 : 1 : 1) as growth medium having a bulk density of 1.1g cm⁻³. These seedlings were kept to re-establish for 25 days before application of water deficit treatments. Length of the seedlings was maintained to a height of 10 inches. Seven treatments containing a control and six drought levels were maintained throughout the experiment (April to October). In this experiment, 100 percent field capacity was taken as control (T₀) while 85% (T₁), 70% (T₂), 55% (T₃), 40% (T₄), 25% (T₅) and 5% (T₆) field capacity were the different drought levels used in this experiment. The experiment was laid out in Completely Randomized Design (CRD) having three replications.

Physical and chemical characteristics of growing media

Physical and chemical characteristics of the growing media (pH 7.92) used as media (sand, silt and leaf compost) for the experiment were calculated in the form of media saturation paste in which soil was soaked with distilled water and allowed to stand over-night. To calculate saturation percentage (SP)

a 50 g weight of saturated paste was dried in oven and saturation percentage was calculated by using method [Method 27a, United States Salinity Laboratory Staff, 1954].

$$SP = \frac{\text{Loss in weight on oven drying (g)}}{\text{Oven dried soil weight (g)}} \times 100$$

For the determination of field capacity, samples of 100 g of each of the media were taken at the time of filling pots. These samples were incubated at 105°C for 24 hours. This oven dried soil was weighed and average for the determination of total moisture contents of the soil at the time of transplanting silvery. Field capacity was calculated by the formula:

$$FC = \frac{\text{Saturation percentage}}{2}$$

Electrical conductivity (ECe) of saturation extract was noted with conductivity meter (Jenway Model-4070) after calibrating it with 0.01 N KCl solutions and cell constant was calculated:

$$ECe = \frac{1.4118}{EC \text{ of } 0.1N \text{ KCl}}$$

ECe = 6.83 ds m⁻¹ dried at 105°C to a constant weight

Morphological attributes

The total number of leaves/plant, number of flowers/plant and number of lateral branches were counted manually at every 10 days' interval during the whole experiment and average was computed. Leaf firing percentage was estimated as the total percentage of chlorotic leaf area. Percentage of leaf firing was visually assessed every 2nd day [Beard and Sifers 1998] at 11:00 a.m. throughout the experiment period by a gauge of 0 to 100% (0% with no leaf firing and 100% with complete leaf firing). Leaf area index (LAI) was calculated after 30 days by a leaf area meter (LI-COR, Model 3100, USA) as the ratio of leaf area to land area. Plant quality is a composite of several characters including wilting, burning or discoloring stress sign, insect pest damage to plant, etc. Plant quality was rated in number using a scale ranging from 1 to 6 with 1 as dead or mostly dead plants, 6 as excellent good plant growth with no sign of stress and or disease. On the compilation of experiment plants were taken out of pots; fresh and dry

weight of shoots and root, root-shoot ratio was recorded following the methods of Mahmood et al. [2008].

Physiological attributes

The physiological attributes were determined after 30 days from application of treatments. The water use efficiency (A/E) was calculated by dividing photosynthetic rate [net CO₂ assimilation rate (A)] by transpiration rate (E). For leaf water potential three fully expanded flag leaves were sampled per replicate from each treatment between 1100 and 1300 hours. It was measured using pressure chamber (Model OSK2710, OGAWA Seiki Japan). Measurement of photosynthetic rate (A), stomatal conductance (g_s), transpiration rate (E), and sub-stomatal CO₂ concentration/Internal CO₂ conc. (C_i) were measured at 11:00 a.m. to 12:00 p.m. from a full expanded 2nd leaf of every plant through portable infrared gas analyzer (IRGA) (Model LCA-4 ADC, Analytical Development Company, Hoddesdon, England). Membrane stability (electrolyte leakage) was measured by method adopted by Blum et al. [1988]. Six leaf pieces of uniform size were immersed in DD water for 12 h to measure electrical conductivity (EC₁) by using EC meter. Samples were then kept in boiling water for 1h and then let it cooled down to room temperature. The electrical conductivity (EC₂) of these tissues was again recorded. Membrane stability was determined as the ratio between EC₁ and EC₂. Leaf temperature was taken by using IRGA. Temperature of 5 attached leaves was taken from each plant and mean was calculated. For chlorophyll determination leaf sample of 0.5 g was grinded in 5 mL of 80% acetone. Poured it in cuvettes and read at 663 and 645 OD's using UV-spectrophotometer (UV-4000) values were substituted in the following formula below:

$$A = \frac{[\{ 0.0127(OD663) - 0.00269(OD645) \} \times 100]}{0.5} = \frac{\text{mg}}{\text{g}} \text{ of fresh weight}$$

$$A = \frac{[\{ 0.0229(OD665) - 0.00468(OD663) \} \times 100]}{0.5} = \frac{\text{mg}}{\text{g}} \text{ of fresh weight}$$

Statistical analysis

Data pertaining to various morphological and physiological attributes were analyzed using ANOVA techniques [Steel et al. 1997]. The means values were compared with least significance difference test (LSD) at 5% level of probability.

RESULTS

Results obtained on weight of different plant part depicted that at 100 percent field capacity (control) plant gained the maximum biomass compared with other treatments. Treatment means were highly significant with each other. For shoot fresh weight, maximum weight was obtained at minimum drought levels (85–40%) as well as fresh weight of shoot severely decreased from 25 to 5% drought level (tab. 1). Results obtained for dry weight of shoot showed that it also decreased with increasing drought levels and minimum drought level gave maximum dry weight of shoot. Results regarding fresh weight of root depicted that maximum root fresh weight was obtained with 100% field capacity while minimum root fresh weight was obtained with maximum level of drought. Field capacity at 100% level yielded highest dry weight of roots compared with other treatments (tab. 1). Maximum root length was obtained with minimum drought and the length of roots tends to decrease with severity of drought (fig. 1).

In the present study, silvery showed similar results of root length at control and 85% field capacity. Results obtained for number of lateral branches showed that number also decreased with increasing drought level and 100% field capacity gave maximum number of branches compared to other drought levels as presented in the Figure 1.

Results regarding plant height depicted that maximum plant height was obtained with 100% field capacity it gradually decreased with severity of drought. Minimum plant height (25.98 cm) was observed in most severe drought treatment (at 5% field capacity). As far as number of flower per plant was concerned, no significant decrease was observed up to 40% field capacity and it remained almost constant but after that a significant decrease in number of flowers were recorded (fig. 1).

Results regarding flower quality revealed that flowers maintained their quality up to 40% field capacity drought stress and no significant decrease in flower quality was envisaged. Flowers tend to deteriorate with severity of drought and plants grown at 25 and 5% field capacity led to produce flowers of small size and of inferior quality. For leaf parameters, similar trend was observed in this study. The value of these characteristics decreased with increase in drought stress except leaf temperature and leaf firing percentage value of which increased with severity of drought (tab. 2). While the value of number of leaves/

Table 1. Effect of drought levels on fresh and dry weight of root and shoot of *L. frutescens*

Field capacity	Shoot fresh weight (g)	Shoot dry weight (g)	Root fresh weight (g)	Root dry weight (g)
Control	35.52a	12.78a	2.83a	1.29a
85%	35.52a	11.99b	2.77a	1.28a
70%	34.68a	11.93b	2.57a	1.17b
55%	32.98a	10.20b	2.26b	1.10b
40%	31.86a	9.65c	2.15b	1.02b
25%	24.56b	8.78c	1.76c	0.83c
5%	14.25c	8.12c	1.12d	0.79c
LSD Value	1.0983	0.3652	0.0884	0.0312

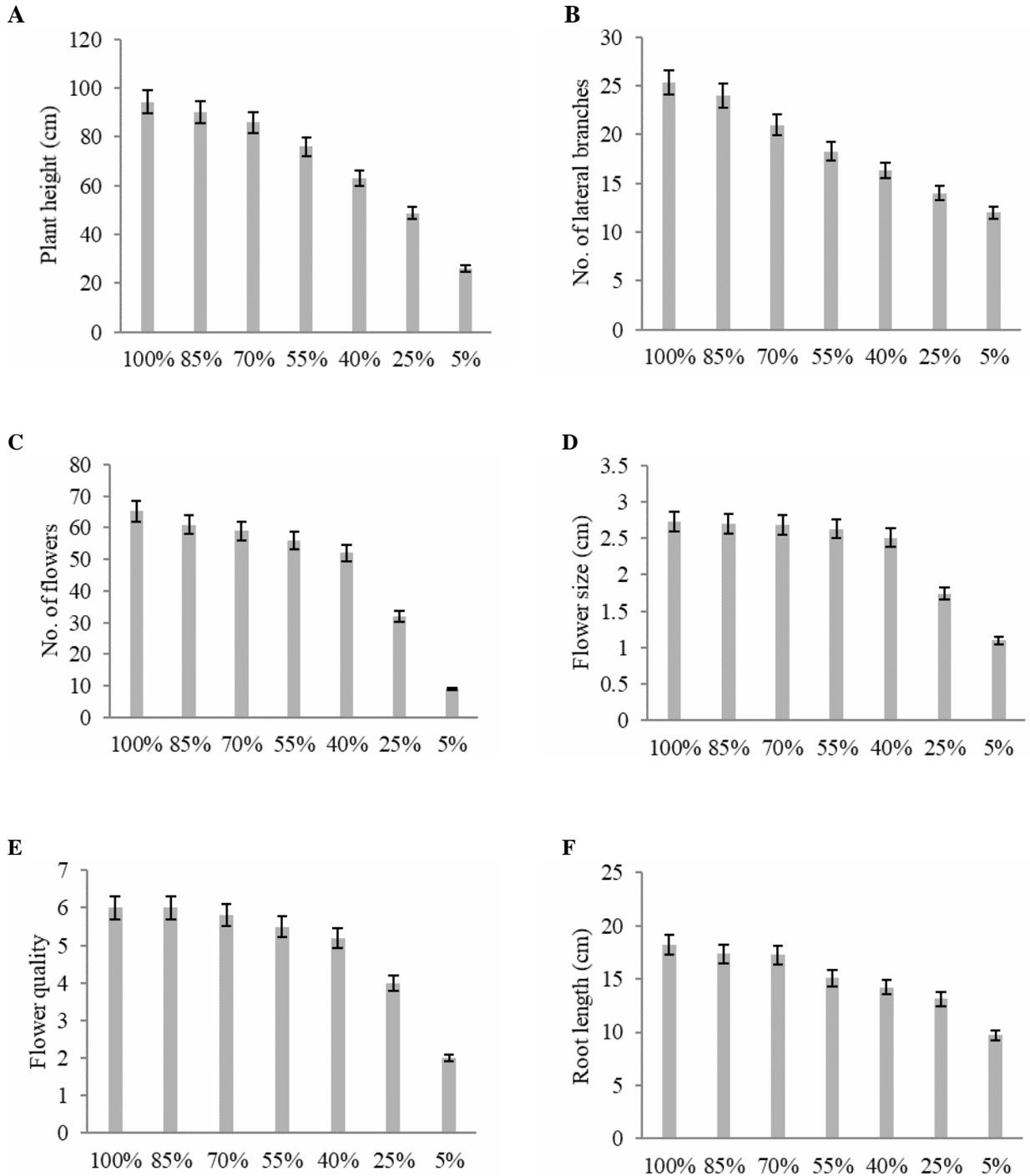


Fig. 1. Effect of drought on different morphological characteristics of *L. frutescens*: A) plant height, B) number of lateral branches, C) number of flowers, D) size of flowers, E) flower quality and F) root length

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Table 2. Effect of drought levels on different leaf characteristics of *L. frutescens*

Field capacity	No. of leaves/plant	Leaf area (cm ²)	Leaf thickness (μ)	Leaf firing (%)	Leaf area index	Leaf temperature (°C)
Control	475.00a	2.09a	340.42a	6.33a	2.18a	18.33d
85%	454.00a	2.07a	338.36a	9.66a	2.08a	19.66d
70%	448.33a	2.06a	323.76b	14.33a	1.92a	23.00c
55%	441.00a	1.89a	313.41b	17.33b	1.80b	24.33c
40%	413.00b	1.66b	288.01c	23.00b	1.72b	28.66b
25%	323.33c	1.08c	223.51d	48.66c	1.12c	30.76a
5%	188.66d	0.89d	201.08e	80.23d	0.92d	30.89a
LSD Value	23.809	0.067	11.231	8.190	0.122	1.050

Table 3. Effect of drought levels on different physiological attributes of *L. frutescens*

Field capacity	Water use efficiency (A/E) (mmol H ₂ O·mmol ⁻¹)	Leaf water potential (Mpa)	Photosynthesis rate (μmol m ⁻² s ⁻¹)	Cell membrane stability (%)	Total chlorophyll contents mg g ⁻¹	Stomatal conductance (gs) (mol m ⁻² s ⁻¹)
Control	4.13a	0.21a	60.0a	61.05a	1.65a	0.55a
85%	4.04a	0.18a	58.0a	60.66a	1.59a	0.53a
70%	3.32a	0.13a	51.0b	58.01a	1.54a	0.50a
55%	2.42b	0.10b	48.0b	51.33b	1.43b	0.47b
40%	2.33b	0.09c	46.0b	48.66b	1.38b	0.39b
25%	1.21c	0.07c	35.0c	31.33c	1.09c	0.21c
5%	0.32d	0.03d	22.0d	23.66d	0.87c	0.15d
LSD Value	0.1598	0.0092	3.3546	4.5299	0.0611	0.0192

Table 4. Correlation of morphological and physiological attributes in *L. frutescens*

	Physiological attributes				
	Water use efficiency(A/E) (mmol H ₂ O·mmol ⁻¹)	Leaf water potential (Mpa)	Photosynthesis rate (μmol m ⁻² s ⁻¹)	Cell membrane stability	Total chlorophyll contents
Fresh wt. of shoot	0.053 (0.00)**	0.123 (0.03)*	0.222 (0.002)**	0.112 (0.01)**	0.023 (0.02)**
Root length	0.012 (0.002)**	0.022 (0.001)**	0.0034 (0.00)**	0.231 (0.023)*	0.034 (0.00)**
Leaf area	0.014 (0.0042)*	0.142 (0.000)**	0.125 (0.00)**	0.042 (0.000)**	0.345 (0.002)**
Leaf temperature	0.0056 (0.000)**	-0.042 (0.001)**	-0.245 (0.001)**	-0.214 (0.00)**	-0.347 (0.032)*

* P < 0.01, ** P < 0.05

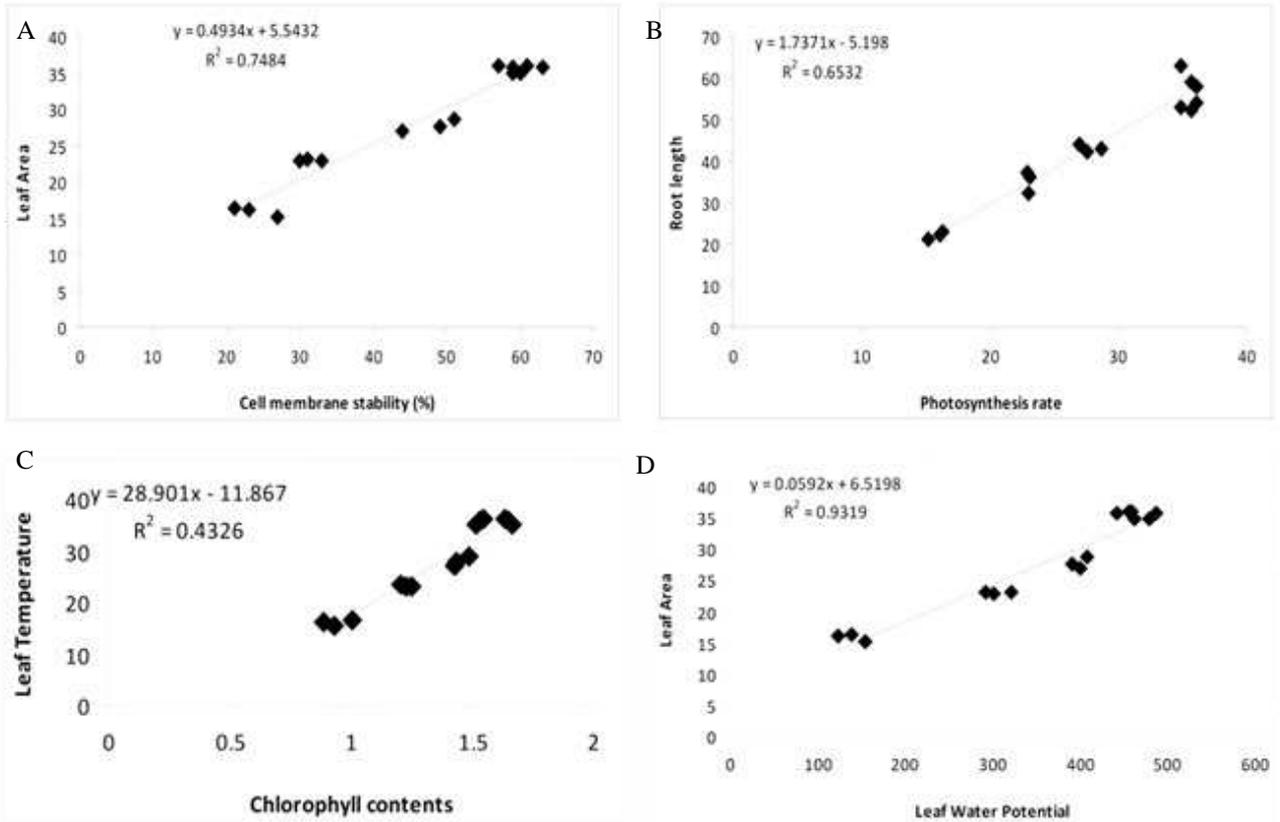


Fig. 2. Regression analysis of A) leaf area and cell membrane stability, B) root length and photosynthesis rate, C) leaf temperature and chlorophyll contents and D) leaf area and leaf water potential of *L. frutescens*

plant (475), leaf area (2.09 cm^2) and leaf area index (2.18) was highest at 100% field capacity (control) followed by the output at 85% of field capacity. In case of different physiological attributes, present data showed better results at 100% field capacity as compared to other water deficit treatments. Minimum values of all physiological parameters were obtained as level of drought increased (tab. 3). When the field capacity was maintained at 100% there were maximum results for water use efficiency ($4.13 \text{ mmol/mmH}_2\text{O}$), leaf water potential (0.21 Mpa), photosynthesis rate (60.00), cell membrane stability and total chlorophyll contents (1.65). Minimum rate of these attributes was achieved at field capacity of 5%.

Data regarding relation of various morphological parameters with morphological traits of silvery also showed significant results. Positive correlation was exhibited among most of the attributes but leaf temperature was negatively correlated with leaf water potential, photosynthesis rate, cell membrane stability, and chlorophyll contents (tab. 4). Moreover, regression analysis between various morphological and physiological attributes showed the predictive power of the model. Significant results were obtained for leaf area and cell membrane stability ($R^2 = 0.7484$), root length and photosynthesis rate ($R^2 = 0.6532$), leaf temperature and chlorophyll contents ($R^2 = 0.432$) and leaf area and leaf water potential ($R^2 = 0.9319$) as demonstrated in the Figure 2.

DISCUSSION

For ornamental plants, the scarcity of water is a severe constraint which effects its utilization in landscape [Riaz et al. 2010, Riaz et al. 2013]. Water stress caused the morphological and physiological modifications in *L. frutescens* plants. It is important to state that different stress conditions in plants brought different structural and functional changes for their better adoptability in these settings [Nadeem et al. 2012, Younis et al. 2013]. Reduction in *L. frutescens* plant growth, height and spread were revealed under present experimental conditions which are similar with findings of different researchers. Tsialtas et al. [2001] observed one-third reduction in plant fresh weight of the *Ziziphus rotundifolia* under drought conditions.

Higher field capacity levels stimulated to increase root dry weight and decrease in root dry weight was recorded under severe drought stress [Wullschlegler et al. 2005]. Nadeem et al. [2012] reported the reduction in the growth of the roots due to low water supply. It corresponds with the findings of Wang and Yamauchi [2006] who argued that root growth is retarded in dry soil conditions, but these conditions will not affect shoot growth whereas in *Populus* species drought stress increased the root length. It shows the competitive behavior of the bushes for the survival as Schuppler et al. [1998] pointed out that the amount of moisture is generally higher in the deep layer of soil this may leads to extension of the roots for water absorption, because of changes in cell wall in the apical part of the root making it more extensible. On the other hand, penetration of the roots hindered with the increasing dryness in the upper layers. Other argument is that under high water stress conditions, cell elongation may be suppressed due to water flow interruption from the xylem to elongating cells in higher plants. Similar results were explained by Riaz et al. [2016] where they report significant decrease in lateral branches with higher levels of drought stress. El-Juhany and Aref [2005] also observed reduction in the number of leaves of *Conocarpus* species under drought stress.

As in present trial, decrease in plant height was observed under severe water deficit conditions which were also recorded by [Kanwal et al. 2012]. In citrus seedlings, 25% reduction in the plant height was recorded under water deficit conditions [Wu et al. 2008]. Significant decrease in plant height under drought conditions was reported in *Leucophyllum* species [Stabler and Martin 2004], *Conocarpus* species [El-Juhany and Aref 2005, Riaz et al. 2016] and lady's finger (*Abelmoschus esculentus*) [Sankar and Jaleel 2008]. In present experiment, short plant height was also recorded that may be attributed due to low turgor pressure which suppresses cell growth and cell expansion greatly. It is important that osmotic pressure regulates the cell turgor that is essential for plant under severe water stress [Shao et al. 2008, Younis et al. 2014]. Other possible reason for low plant height under water stress is related with more leaf senescence and decline in the cell enlargement [Bhatt and Rao 2005].

Decrease in leaf area index which in turns lowers the photosynthesis and photosynthetic active radiation under prolong drought conditions [Fleisher et al. 2008]. Furthermore, expansion in leaf area largely related to leaf turgor, plant temperature and as well as assimilating supply for growth [Youssefi et al. 2011]. But under drought leaf area gets shorter so that less leaf area exposure to sun which reduces the transpiration and photosynthesis rate. One of the reasons for reduction in photosynthesis rate was explained by Pospisilova et al. [2000]. They stated that extreme water deficiency causes reduction in photosynthetic activity in mesophyll cells that result reduction in electron transport chain and carboxylation processes, and induces ultrastructural modifications in chloroplast. Water use efficiency of *L. frutescens* was found significantly higher as compared to *Spirea vanhouttei* and *Viburnum tinus*. Prolonged water deficit adversely influences the water contents in leaf, water-use efficiency and photosynthesis [Egilla et al. 2005]. The extreme drought stress adversely affects photosynthesis activity in mesophyll cells and result reduction in electron transport chain and carboxylation processes and as well as makes ultra-structural modifications in chloroplast cells [Pospisilova et al. 2000, Mansoor et al. 2015].

In addition to above mentioned effects of drought there is also destructive effect of drought on cells which causes loss of turgor pressure and cell expansion resulting to the production of Reactive Oxygen Species (ROS) during oxidation stress. ROS cause the denaturing of structural and vital organelles of plants and biochemical compounds necessary for the life process of the plants [Karthikeyan et al. 2007]. Blum [2005] stated that in crop plants drought tolerance showed that genotype diversity in plant retrieval after long dryness, as a degree of tolerance, was recorded positive correlated with water status within plant. In the present study, negative correlation between leaf temperature and other traits was exhibited. One of the possible reasons for a negative association between drought resistance and yield potential can be due to high yield and a large sink create a burden in shoots for its turgor maintenance and water status as reported in cereals [Blum et al. 1988]. In another study, Tahir and Mehid [2001] found that in sunflower water deficiency reduced not only the head diameter but also achene weight and yield/plant. Negative relationship between head diameter, shoot weight and fresh root weight was observed, whereas, a positive correlation between shoot dry weight and number of achene/plant was recorded.

CONCLUSION

This study highlights the potential use of *L. frutescens* under different water regimes in urban landscape. This species tolerates drought conditions up to 40 percent field capacity and no significant change in morphological characteristics were observed but under severe water stress conditions plant and flower quality start decreasing. The plant of this species remains alive and produces flowers even at 25 and 5 percent field capacity. Hence, *L. frutescens* proved to be a potential plant species for urban planting and landscape compared with other landscape plants due to its low water requirement.

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