JOURNAL OF PURE & APPLIED MICROBIOLOGY, Nov. 2014. Vol. **8**(Spl. Edn. 2), p. 399-408

Effects of Water Stress and Salinity on the Growth of *Hibiscus tiliaceus* Trees

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(Received: 03 August 2014; accepted: 20 October 2014)

Hibiscus tiliaceus L. is the last tree species that has been spread in Riyadh City lately because of its ability of tolerate the harsh environmental conditions and due to the beauty of its large yellow flowers. In the present study, four months old seedlings of _Hibiscus tiliaceus_ were subjected to water stress (through withholding irrigation) and salinity (by increasing salt concentration in the irrigation water). Four treatments were applied to the plants growing in the pots included two levels of watering; every other day (well-watered treatment) and every 5 days (water stress treatment), and two levels of salinity (sodium chloride dissolved in the irrigation water); mainly tap water (0.97 dsm^{-1})(low salt concentration treatment) and 16 dsm^{-1} (high salt concentration treatment). The results showed that water stress decreased stem height and diameter, number of branches, total leaf area and specific leaf area. While increasing salt concentration caused decreases in stem height and diameter, number of leaves, total leaf area, leaf dry weight and specific leaf area. Both water stress and high salt concentration treatment resulted in a marked decrease in relative leaf water content and a significant increase in proline content in the leaves of _Hibiscus tiliaceus_ seedlings. These results indicate that this species is tolerant to water and salinity stresses and suits the environmental conditions of Saudi Arabia.

Key words: *Hibiscus tiliaceus* L., water stress, salinity, growth, proline.

Saudi Arabia has harsh environmental conditions due to its location in the arid zone. It has a desert climate characterized by extreme heat in summer and very low annual rainfall, with the exception of the southwestern heights. Saudi Arabia is one of the driest countries in the world. The climate in Riyadh City is marked by extremes of temperature, with low humidity throughout the year, particularly in the summer season (El-Juhany and Al-Harby 2013).

A lot of efforts have been done over the last four decades to mitigate theses harsh environmental conditions through establishing gardens and planting different plant species. The afforestation motion in Riyadh has passed through different episodes related to the expanding of the city, the problems that encounter planting and maintaining the plants, withstanding plants to weather and others. As tree planting is at the core of the concept to combat harsh climate, it represents the majority of the plants used for greening of Riyadh City. However, the constraints that encounter planting trees in arid regions are water scarcity and/or its availability over long intervals, low relative humidity, wind loaded with sand and dust, high temperature, sand movement and lake of protection for trees (Aref *et al*., 2006).

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In Saudi Arabia saline water is one of the most frequent environmental stresses that face growing plants (Aref *et al*., 2004). This because groundwater represents more than 90% of water used in agricultural irrigation and it classified as very saline water (Falatah *et al*., 1999). Due to the scarcity of water in Saudi Arabia groundwater is considered the main source of agriculture irrigation water. However, groundwater is known that it contains high salt concentration (Al-Omran *et al*., 2005). Al-Matroud (2003) reported that salinity of groundwater from Riyadh Region (Central part of Saudi Arabia) has electrical conductivities (EC) ranged between 1.34 and 7.84 dS m-1 and dominated by sodium chloride cations.

Water supply is the most important environmental factor that determines the productivity plants. This is due to the vital role of water in the plant's life (Kramer 1959). Responses of forest tree species to water stress have widely been documented (*e. g*. Kozlowski 1982; Ibrahim *et al.*,1997 and 1998; Aref and El-Juhany 1999; El-Juhany and Aref 1999, Aref and El-Juhany 2001; EI-Juhany, 2003; El-Juhany *et al*., 2008b). On the other hand, the adverse effects of increasing salinity level on the growth of tree species has been extensively reported (*e. g*. Kelsey and Hootman 1990; Omran *et al.*,1996; Fostad and Pedersen 2000; Tomar *et al.*,2003; El-Juhany *et al*., 2008a). Many researches have been done with either water stress or salt stress separately, probably as a matter of convenience. However, the most realistic test would be to combine salt and water stress, as this would simulate conditions that are often found in the field under arid conditions. The clear separation between salt and water stress that is a feature of the physiological literature seems highly artificial (McCree 1986).

Hibiscus tiliaceus L. is the last tree species that has been spread in Riyadh City lately because it has the ability of tolerate the harsh environmental conditions and due to the beauty of its large yellow flowers.It is a fast-growing small, evergreen polymorphic tree that typically ranges from 4 to 12 m in height and 15 to 20 cm d.b.h. The tree most often has a short, crooked trunk with a broad crown of widely spreading and crooked branches. It may also grow either in a prostrate, spreading form, or more rarely in a taller, straighter form that may reach 20 m in height (Little and Skolmen 1989, Wagner *et al*.,1990).

H. tiliaceus exhibits a generalist strategy with regard to salinity tolerance (Santiago *et al*., 2000) and, can tolerate saline conditions and presumably would do well even with brackish irrigation water (Elevitch and Thomson 2006)**.** On the other hand, once established, the tree handles drought very well (Elevitch and Thomson 2006). It is recorded to be fairly tolerant to drought (Achigan-Dako 2011). It seems that *H. tiliaceus* has a wide environmental tolerance and can withstand saline infertile soils and drought.

As there were no studies on the suitability and performance have been done for this plant species in Saudi Arabia, the present work aims at clarifying the suitability of *H. tiliaceus* to water stress and salinity conditions that prevail in most of Saudi Arabia cities.

MATERIALS AND METHODS

Plant material

Four months old seedlings of *Hibiscus tiliaceus* L. grown from cuttings were used in the present study. The seedlings remained in the greenhouse under 25/20°C day/night and received watering every other day for four weeks before they were subjected to treatments.

Statistical design and treatments

16 pots containing one plant each were arranged in the greenhouse using a complete randomized design with four replications in a factorial arrangement. Four treatments were applied to the plants growing in the pots included two levels of watering; every other day (well-watered treatment) and every 5 days (water stress treatment), and two levels of salinity (sodium chloride dissolved in the irrigation water); mainly tap water (0.97 dsm^{-1})(low salt concentration treatment) and 16 dsm^{-1} (high salt concentration treatment). Sodium chloride was used to prepare solutions varied in their salinity level because it is easier in application and control. Salinity studies are almost exclusively conducted with treatmentsalt concentrations, usually NaCl or fixed ratios of mixed salts as it is expedient and easily managed (Grieve and Shannon 1999).

Harvesting and growth measurements of the trees

Total stem height (from the soil surface to the top of the plant) was measured using a ruler and stem diameter were measured at a point on the stem spaced ten centimeter from the soil surface using a small tree caliper. Total leaf area of each tree was scaled using leaf area scanner (Hayshai Denkoh Co., LTD. Tokyo, Japan).

Specific leaf area was calculated by dividing the total leaf area for each plant on its leaf dry weight ($cm^2 g$ leaf dry $weight^{-1}$). The root system of each seedling was removed from the soil by hand and washed. All samples were dried in an oven at 70°C for 48 hours.

Determining leaf relative water content (RWC)

Determining leaf relative water content was done for each tree in the experiment before each watering time according to Barrs (1968), through taking three leaves and quantifying their fresh weight then placed them in distilled water for 24 hours to saturation. Thereafter, saturated leaves were weighed and placed in the oven at 70ºC for 48 hours then their dry weight was measured. Leaf relative water content was calculated as follows: RWC = (FW – DW) / (SW – DW) × 100 where RWC = leaf relative water content, FW = leaf fresh weight, SW = leaf saturated weight and, DW = leaf oven dry weight.

Quantifying the proline content in the leaves

The proline content of the leaves was determined according to Bates *et al*. (1973). Approximately 0.5 g of the dried leaves was ground, homogenized in 10 mL of sulfosalicylic acid and filtered through a filter paper. Then, 2 mL of the filtrate was incubated with 2 mL of acidic ninhydrine and 2 mL of glacial acetic acid for one hour at 100°C. The reaction was stopped by transferring the samples into an ice bath to cool. The reaction mixture was extracted with 4 mL of toluene and absorbance was read at 520 nm in spectrophotometer against toluene blank.

The concentration of proline was calculated according to the formula:
[(ml toluene) ×(µg proline/ml)]/(g sample × 2/3) = µg proline/g dry weight.

Statistical analysis

Data were statistically analyzed through analysis of variance procedure using CoStat Statistical Software (1990). Means of different source of variation were distinguished by Duncan's Multiple Range Test at $P<0.05$ (Steel and Torrie 1982). Data of dry matter partitioning were angular transformed before analysis.

RESULTS

Increasing the interval between watering times caused significant decreases in the growth of *Hibiscus tiliaceus* seedlings in greenhouse. The means of their stem diameters and heights decreased by 20% ($P<0.001$) and 25% ($P<0.0001$), respectively (Table 1). There were reductions in the number of branches and total plant leaf area by 45% ($P<0.01$) and 22% ($P<0.01$), respectively (Table 1).

Increasing salt concentration in irrigation water caused decreases in both means of stem diameters and heights by 22%, ($P<0.01$) and ($P<0.0001$), respectively (Table 1). There were also decreases by 18, 34 and 13% in the number of leaves ($P<0.01$), total leaf are ($P<0.001$) and leaf dry weight ($P<0.01$), respectively, while total root length decreased by 4.2% ($P<0.01$) (Table 1). Specific leaf area decreased due to water stress ($P<0.01$) and salinity ($P<0.0001$) by 14 and 22%, respectively (Fig. 1).

Relative water content in the leaves was reduced significantly ($P<0.0001$) in both water stress and high salt concentration treatments and by 42 and 29%, respectively (Fig. 2).

Proline content in the leaves increased significantly in both water stress and high salt concentration treatments ($P<0.0001$). It increased by 93% in water stress treatment and by 39% in high salt concentration treatments (Fig. 3). There were interactions between watering and salt concentration treatments ($P<0.0001$) indicated changing the magnitude of the effects of each on both relative water content and proline content in the leaves with the presence of the other.

DISCUSSION

It is well known that both water deficit and increase salt concentrations (either in irrigation water or in the soil) cause decreases in the growth of plants. However, the response of plants to these environmental stresses varies according to the magnitude of these stresses and to the ability of the plant to tolerate such stresses.The plants that tolerate high salt concentration are called halophytes. They are remarkable plants that tolerate salt concentrations that kill 99% of other species (Flowers and Colmer 2008).

Table 1. Effects of water stress and high saline irrigation on the growth of *Hibiscus tiliacius* L. seedlings after three month of treatment. WW, LW, LS and HS are well-water supply, low water supply, low and, high salt concentration, respectively. Values are means ± SE of eight observations

Trait	Water supply	Salt concentration LS	Salt concentration HS	Mean
Stem height (cm tree^{-1})	WW	85.1	62.1	73.6^a
	LW	60.1	50.4	55.3^b
	Mean	72.6^a	56.3^b	±1.87
Stem diameter (mm tree^{-1})	WW	8.4	5.8	7.1^a
	LW	5.4	4.9	5.7^b
	Mean	6.9^a	5.4^b	±4.77
Total leaf area (cm^2 tree^{-1})	WW	714.8	497.0	605.9^a
	LW	579.7	361.6	470.7^b
	Mean	647.3^a	429.3^b	±51.7
No. of leaves (leaf tree^{-1})	WW	25.0	14.0	19.5^a
	LW	18.0	11.5	14.8^a
	Mean	21.5^a	12.8^b	±3.3
No. of branches (branch tree^{-1})	WW	7.8	5.0	6.4^a
	LW	3.8	3.3	3.5^b
	Mean	5.8^a	4.1^a	±1.04
Leaf dry weight (g tree^{-1})	WW	15.5	13.0	14.3^a
	LW	13.6	12.2	12.9^a
	Mean	14.5^a	12.6^b	±0.69
Total root length (cm tree^{-1})	WW	51.9	49.7	50.8^a
	LW	41.8	40.1	41.0^b
	Mean	46.9^a	44.9^b	±4.34

†Means followed by different superscripted letters in every two consecutive horizontal (effects of salinity stress) or vertical (effects of water stress) boxes are significantly different at probability level ≥.05 according to Duncan's Multiple Range Test

Due to the scarcity of water in Saudi Arabia groundwater is considered the main source of agriculture irrigation water. Falatah *et al*., (1999) found that the salinity of groundwater used for irrigation in eight important agricultural areas in Saudi Arabia represented by the electrical conductivity (EC, dS m^{-1}) vary from area to another, and ranged between 0.17 and 29.31 dS m^{-1}.

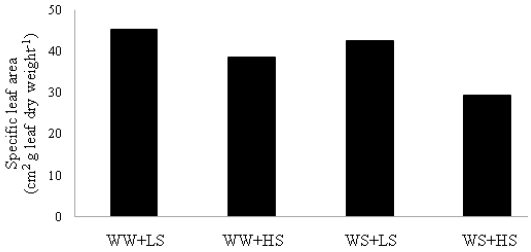

Fig. 1. Specific leaf area (cm^2 g leaf dry weight^{-1}) of *Hibiscus tiliaceus* seedlings grown under four treatments combination: WW+LS= well-watered with low salt concentration; WW+HS= well-watered with high salt concentration; WS+LS= water stressed with low salt concentration and WS+HS= water-stressed with high salt concentration

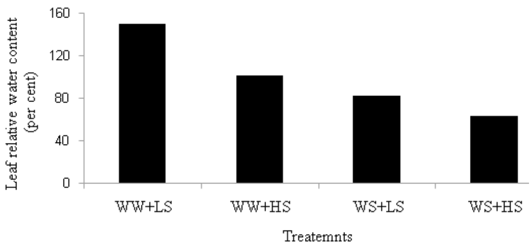

Fig. 2. Relative leaf water content (per cent) of *Hibiscus tiliaceus* seedlings grown under four treatments combination: WW+LS= well-watered with low salt concentration; WW+HS= well-watered with high salt concentration; WS+LS= water stressed with low salt concentration and WS+HS= water stressed with high salt concentration

Hibiscus tiliaceus is a mangrove associate species so that it grows well under drier conditions and in a variety of soils and it can also stand brackish water and is tolerant of salt spray, and therefore it is an excellent species for coastal areas (Fryxell 2001).Mangrove associates represent transitional lifestyles between terrestrial plants and true mangroves and are ideal subject material to study the mechanism of adaptive evolution in plants. *Hibiscus tiliaceus* is such a species that has two ecotypes adapting to littoral and terrestrialhabitats, respectively (Shan *et al*., 2008). However, *Hibiscus tiliaceus* is not a true mangrove and does not possess any salt secreting structures (Youssef 2007).

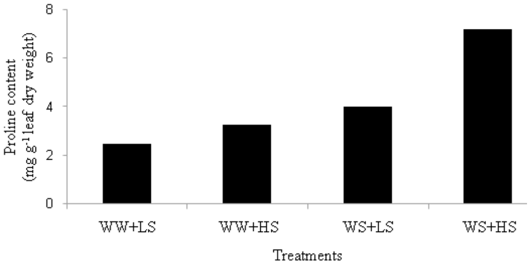

Fig. 3. Proline content (mg g^{-1} leaf dry weight) of *Hibiscus tiliaceus* seedlings grown under four treatments combination: WW+LS= well-watered with low salt concentration; WW+HS= well-watered with high salt concentration; WS+LS= water stressed with low salt concentration and WS+HS= water stressed with high salt concentration

The results of the present work showed that some morphological growth traits of *Hibiscus tiliaceus* seedlings decreased due to increasing the interval between watering times or increase salt concentrations. In both treatments, there were decreases in stem diameter and height and total leaf area. Decreasing stem diameter and height of woody species under water deficit has been reported by many researchers (*e. g*. Aref and El-Juhany 1999; El-Juhany and Aref 1999; El-Juhany 2003; El-Juhanyand Aref 2005; El-Juhany*et al*., 2008c; Zahidi *et al*., 2013). The adverse effects of salinity on stem diameter and height in the present study concurs with other results (*e. g*. Bernstein *et al*., 2001; El-Juhany and Aref 2005; El-Juhany *et al*., 2008; Mansour *et al*., 2010; Elfeel and Bakhashwain 2012; Ali *et al*., 2013).

Decreasing total leaf areadue to water stress is known as a mechanism by which plant preserve water inside its tissues. This decrease may be achieved through inhibiting leaf initiation (Kozlowski 1982 and Ibrahim 1995) or decreasing leaf size (Ibrahim *et al*. 1997 and 1998) or accelerating leaf senescence and consequently leaf shedding (Begg 1980) or more than one of them. Similar results on decreased total leaf area due to water stress were reported (*e. g*. Ibrahim *et al*.,1997 and 1998,Aref and El-Juhany 1999, El-Juhany and Aref 1999, El-Juhany and Aref 2005, Aref and El-Juhany 2005,El-Juhany *et al*., 2008b).In high salt concentration treatment,total leaf area decreased by 34% and was more pronounced than in water stressed treatment (22%). Decreasing total leaf area due to salinity has been found by others (*e. g*. Youssef 2007; El-Juhany *et al*. 2008a; Ziaf *et al*., 2009; Mansour *et al*., 2010; Elfeel and Bakhashwain 2012; Ali *et al*., 2013). The results of the present study show that the decrease of total leaf area in high salt concentration treatment was due to a decrease in the number of leaves per plant. Other results showed leaf area was not affected by salinity stress (Dolatabadian *et al*., 2001).

In the present study, the number of leaves was not significantly affected by water stress but decreased by 18% in high salt concentration treatments. This result concurs with others (El-Juhany and Aref 2005; El-Juhany *et al*., 2008; Sohail *et al*., 2009). In contrast, other reports showed that the number of leaves per plant decreased due to water stress (Ibrahim *et al*., 1997 and 1998; Aref and El-Juhany 1999; El-Juhany and Aref 1999; El-Juhany 2003; El-Juhany and Aref 2005; Tiwari *et al*., 2013).

On the other hand, the reductions of the number of branches per tree were 45 and 29% in water stress and high salt concentration treatments, respectively. However the reduction in the later was not statistically significant. Decreasing number of branched in water stress was previously reported (*e. g*. Osório*et al*. 1998; El-Juhany and Aref 2005; Bilibio *et al*. 2011). Other researchers found number of branches per plant decreased with increasing salt concentration (*e. g*. El-Juhany and Aref 2005; Razmjoo *et al*., 2008; Mansour *et al*., 2010; Muhammad and Hussain 2010; Ali *et al*., 2013 in salinity).

Although leaf weight of *Hibiscus tiliaceus* seedlings decreased by 13% in water stress however, this was not statistically significant, while its decrease under saline irrigation was significantand accounted for 10%. This result concurs with other findings (*e*. *g*. El-Juhany and Aref 2005; El-Juhany *et al*., 2008a; Elfeel and Bakhashwain 2012). Similar trend was observed for root length of *Hibiscus tiliaceus* seedlings in the present study, where it decreased under saline irrigation while did not so in water stress treatment. Total root length of the seedlings decreased under water stress and by 19%.This concurs with results obtained from previous studies (*e*. *g*. Aref and El-Juhany 1999; Bilgin *et al*., 2008; El-Juhany *et al*., 2008b; Shamsizadeh *et al*., 2014). This seems true as the rate of root elongation is highly dependent on available water because root growth is in part a hydration process (Gingrich and Russell, 1956 and 1957).

In the present study, specific leaf area (SLA) decreased under both water stress and high salt concentration conditions, but its decrease due to salinity was larger than in water stress. Specific leaf area (SLA) is an important variable connecting dry matter production with leaf area and photosynthesis. Decreasing SLA due to water stress has been reported (*e*. *g*. Ibrahim *et al*.1997 and 1998; El-Juhany and Aref 1999; Khurana and Singh 2000; El-Juhany and Aref 2005; Rodríguez *et al*. 2005; Wu *et al*., 2008; El-Juhany *et al*., 2008b; Stanton and Mickelbart 2014). On the other hand, the decreased SLA of *Hibiscus tiliaceus* seedlings due to salinity may be due to the different sensitivity of photosynthesis and leaf area expansion to soil salinity. Similar results were obtained from other studies (*e*. *g*. El-Juhany and Aref 2005; Ziaf *et al*., 2009; Rahimi *et al*., 2011; Elfeel and Bakhashwin 2012). Contradictory, specific leaf area (SLA) increased from 150.0 to 229.9 cm^2 g^{-1} in response to salinity, which indicates the formation of thinner leaves and a higher area per unit of dry weight (Hernández *et al*., 2014).

The above discussed traits represent the morphological plant growth ones and showed moderate decreases either in water stress or under high saline irrigation treatment. However, the two physiological traits measured in the present study (*i*. *e*. leaf relative water content and proline content) were markedly affected in both water stress and salinity. The importance of relative leaf water content comes from the role of water in almost all the physiological processes in the plant. Water deficit affects almost every aspect of plant physiology and morphology (Kozlowski *et al*., 1991). Kozlowski (1982) asserted that water deficit reduces growth both directly through effects on cell turgor and indirectly through intermediation of seed germination, photosynthesis, respiration, mineral nutrition, enzymatic activity, hormone relation and nitrogen metabolism.

Leaf relative water content decreased in water stress by 42% and this concurs with the findings of other studies (El-Juhany *et al*., 2008b; Mousavi *et al*., 2009; Hayatu *et al*., 2014). Leaf relative water content also decreased in high salt concentration treatment and by 29%. Similar results were obtained previously (*e*. *g*. Santiago *et al*., 2000; Ziaf*et al*., 2009; Qin*et al*., 2010; Farouk 2011; Elfeel and Bakhashwain 2012).

Water stress caused a large increase in proline content in the leaves of *Hibiscus tiliaceus* seedlings. This result is in agreement with previous results were obtained for other plant species (*e*. *g*. Sofo *et al*., 2004; Ben-Rouina *et al*., 2006; Kala and Godara 2011; Gaikwad *et al*., 2014). Proline increases proportionally faster than any other amino acids in plants under water stress (Bates *et al*., 1973). Accumulation of proline in higher plants is an indication of disturbed physiological condition, triggered by biotic or abiotic stress condition. Free proline content can increase upon exposure of plants to drought, salinity, cold, heavy metals, or certain pathogens (Abraham *et al*.,2010). On the other hand, proline content in the leaves of *Hibiscus tiliaceus* seedlings increased in high salt concentration treatment and this finding concurs with previous ones (*e*. *g*. Mansour *et al*. 2010; Celik and Atak 2012; Benhassaini *et al*., 2012; Chen and Ye, 2014).

CONCLUSION

The seedlings of *Hibiscus tiliaceus* used in the present experiment were grown in a not fully controlled greenhouse and their morphological growth traits that were measured showed moderate changes (reductions) under the treatments, while the physiological traits measured (leaf water content and proline accumulation) showed large changes. This may suggest that this species is tolerant to water and salinity stresses and suits the normal environmental conditions prevailing in Saudi Arabia.

ACKNOWLEDGEMENTS

This project was supported by the King Saud University, Deanship of Scientific Research, College of Science Research Center.

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