J. Appl. Environ. Biol. Sci., 5(7)43-51, 2015
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ISSN: 2090-4274
Journal of Applied Environmental and Biological Sciences
www.textroad.com

Morphological and Anatomical Responses of *Leucaena leucocephala* (Lam.) de Wit. and *Prosopis chilensis* (Molina) Stuntz to RasSudr Conditions

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Received: March 12, 2015
Accepted: May 30, 2015

ABSTRACT

The objective of this research is to evaluate the effect of salinity stress on morphology and anatomy of two leguminous range plants; *Leucaena leucocephala* and *Prosopis chilensis* plants. The investigated plants were irrigated with tap water (control) and two levels of salinity (3500 and 7500 ppm). Increasing salinity of irrigation water from 3500 to 7500 ppm led to reduction in plant height and stimulated the production of tannins in stems and leaflets of both investigated plants. This study demonstrated the presence of some anatomical changes induced by salinity in *Leucaena leucocephala*, and *Prosopis chilensis* leaflets. These anatomical changes included; presence of thick layer of cuticle, reduction in number of cortex layers and intercellular spaces between palisade cells, increase in the elongation of palisade parenchyma tissue and accumulation of tannin - filled cells in it , in cortical region of stem and also in parenchyma cells of its pith. All these anatomical modifications seemed to be crucial for their survival under salinity stress.

KEY WORDS: anatomical change, *Leucaena leucocephala*, *Prosopis chilensis*, salinity

INTRODUCTION

Salinity is the major environmental factor that currently reduces plant productivity [1]. Salt stress can be a major challenge to plants because it limits agriculture all over the world. Salinity effects are more conspicuous in arid and semi-arid areas where 25% of the irrigated lands are affected by salts [2]. Egypt is a predominantly arid country and the scattered rain showers in the north can hardly support any agricultural crops. As more lands have been salinized by poor irrigation practices, the impact of salinity has become more important. High salt content, especially sodium chloride and sulphates, affects plant growth by modifying their morphological, anatomical [3, 4] and physiological traits [5].Such growth impairment is due to osmotic effects and ionic imbalances affecting plant metabolism [6]. Processes such as seed germination, seedling growth, flowering and fruiting are adversely affected by high salt concentration, ultimately causing diminished economic yield and also quality of production [7]. This situation increases the need for naturally adapted salt tolerant plants, which provide excellent material for investigating the adaptation mechanisms used to tolerate high concentrations of salt [8, 9] that may help plant breeder to evolve salt tolerant plant varieties. Therefore, the present investigation was undertaken to study the effect of salinity stress on morphological and anatomical structure of two leguminous range plants *Leucaena leucocephala* and *Prosopis chilensis* representing salt tolerant plants.

MATERIAL AND METHODS

1. Ecological studies:

Plant material and growth conditions:

Field experiment was conducted at RasSudr Experimental Station of Desert Research Center (DRC), South Sinai. Seeds of the two investigated plant species; *Leucaena leucocephala* and *Prosopis chilensis* were sown in polyethylene bags filled with sand and clay soil (1:1) in December, 2006 under greenhouse conditions of the Desert Research Center, Cairo. After complete emergence of seedlings, irrigation of beds was continued with tap water until transplantation to the field experiment at Ras Sudr Experimental Station, South Sinai governorate in June 2007.

The Meteorological data of temperature, relative humidity and rainfall of RasSudr area during 2008-2009 were obtained from the Applied Agricultural Meteorological Laboratory, RasSudr Experimental Station of Desert Research Center .Chemical and physical analyses of the soil supporting *leuceana* and *prosopis* at RasSudr Station were carried according to Page (1987)[10].

The Meteorological data of temperature and rainfall of RasSudr in South Sinai during 2009 illustrated in Fig.1. The obtained data indicated that; rainfall was 12.95 and 13.15 mm recorded on November and December 2009, respectively. The dry period extended from May to June and from August to September in 2009. The lowest temperature recorded was 6.6°C in January. While, the highest was 42.6 in July. Maximum wind speed was 53.5km/h.

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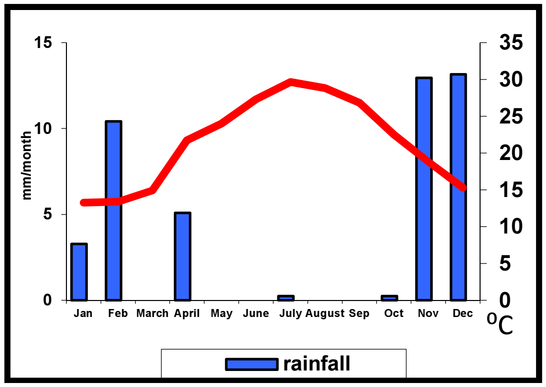

Figure 1. Meteorological data: temperature, relative humidity and rainfall of RasSudr during 2009.

Experimental layout:

The experiment was laid out in split plot design with three replicates. The main plots were occupied by salinity level of irrigation water treatment (tap water and two salinity levels 3500 and 7500 ppm.), pumped from two wells, while the two species were arranged in the sub-plots in rows 1.5 m apart. Salinity levels of irrigation water treatments were continued until June 2010, the date of morphological and anatomical studies.

1. **Morphological Studies:**

Characterization and observations on morphology of the two studied plant species including plant height leaflet and stem morphology as well as flowering and fruiting were recorded and discussed.

2. **Anatomical Studies:**

Leaflet and stem samples from the top of each of the investigated species growing under the three levels of salinity (tap water, 3500 and 7500 ppm) were collected. Leaflets and stems were thoroughly washed and immediately immersed in FAA fixative solution (37% Formalin, glacial acetic acid and 50% ethanol in ratio of 1:1:18, respectively) for 48 h; then they were stored in 70% (v/v) ethanol until use. Samples were embedded in wax and cross sectioned with a manual rotary microtome. The sections are finally mounted on slides using Canada balsam. A hot plate at 40°C was used to dry the slides. The slice slides were examined using image analyzer (inverted microscope Zeiss axiovert25) and light microscope and photographed with an Olympus BX51digital camera.

RESULTS AND DISCUSSION

1.Ecological studies

Results of physical and chemical analyses of the soil utilized in the field experiments indicated that the soil is highly calcareous, the percentage of $CaCO_3$ ranged between 51.21 and 55.58%. Sandy loam in texture with pH 7.7 and ECof 4.77dS/m. Soil soluble cations at the upper (0-30 cm) and lower (30-60 cm) layers were dominated by Ca^{+2} (24 meq^{-1}) and Na^{+} (17.80 meq^{-1}), respectively. Ca^{+2}plays an important role within the plant cell, it is hugely important in primary cell wall structure [11].The anions were dominated by Cl^{-}at bothupper and lower layers, its concentration was 31.20 and 22.50 meq^{-1}, respectively. The soil extract electric conductivity (EC) showed a tendency to decrease with depth from (4.77 to 4.16 dS/m).Also the concentration of $Mg^{+2}$was decreased with depth from11.5 to6.0 meq^{-1}.

2. Morphological Studies:

Leucaena leucocephala: It is an evergreen shrub or tree, which may grow to height 7-20 m with alternate bipinnate leaves, 4-7 pairs of pinnae (4-10 cm long), each with 10 to 20 pairs of lanceolate leaflets (Fig. 2). These are less than 45 mm long. Both leaves and leaflets fold up in response to moisture stress, low temperature and darkness. The flowers are white, in globose heads of about 2 cm diameter. They emerge from the leaf axils at the end of the branches, single or in pairs. The pods are flat, 10-15 cm long pairs [12].

Prosopis chilensis: Single stemmed tree, 3-10 m in Height. Leaves are as long or longer than the raceme. The petiole is 1.5 to 12 cm long with 1 to 3 pairs of pinnae 8 to 24.5 cm long which bear 10 to 29 pairs of leaflets (Fig.3). Leaflets are pale green, with poorly visible veins, 10 to 63 mm long, linear and glabrous with entire margins. They are separated on the rachis by 4 to 12 mm, which is equal to or greater than their width of 1.1 to 3 mm. The flower petals are 3 mm long and hairy within. Stamens are 5 to 6 mm long and the ovary is pubescent. The legume is linear and flattened, about 12 to 18 cm long, 1 to 1.8 cm broad, and 0.6 cm thick with parallel margins. It is nearly straight to sickle shape and contains 20 to 30 seeds [13].

In respect of the effect of salinity stress on the morphology of plants, it can be reported that *Leucaena leucocephala* and *Prosopis chilensis* plants exhibit various alteration in plant height and changes in leaflet and stem morphology when subjected to salinity stress. Irrigation with high level of salinity (7500 ppm) causing reduction in plant height of *Leucaena and Prosopis* in comparing with low level of salinity (3500 ppm), from 2.55 to I.96 m in *Leucaena* and from 3.73 to 2.74 m in *Prosopis*, as a result of reduced cell expansion and cell division [14]. Since high salinity have adverse effect on flowering and fruiting [7], irrigation with high level of salinity causing delay in fruiting of *Prosopis* compared with the plant that irrigated with low level of salinity. Site conditions also have a marked effect on pod production. Where soils are poor, or deficient in any required nutrient, pod production would be expected to be reduced. Soils that are highly alkaline and/or saline may be expected to produce only poor pod yields [15]. Felker *et al* (1984) [16] found that *Prosopis juliflora* and *P.pallida* did not commence fruiting until4-5 years after planting. This may be the result of the higher temperature requirements of these species not being met in sub-tropical USA. Maun (1993) [17] stated that, on average, trees began flowering and fruiting in the third year after planting in Brazil, whereas four years was more common in India [18]. Gomes (1961)[19] found that planting *P. pallida* at the onset of the rainy season resulted in trees that flowered in the second year after planting, whereas other trees flowered in the third year. Wild seedlings are often seen to begin flowering only after 3-6 years. In this study, *Prosopis* irrigated with low level of salinity, started flowering and fruiting in the third year after planting. While *Leucaena* began flowering and fruiting in the first year after planting, indicating no effect of salinity stress on flowering.

Figure 2. Leucaena leucocephal a tree growing in Ras Sudr Experimental Station of Desert Research Center (a), pods(b).

Figure 3. *Prosopis chilensis* tree growing in Ras Sudr Experimental Station of Desert Research Center (a) pods(b).

3.Anatomical Studies:

The cross sections of *Leucaena leucocephala* leaflets showed adaptive mechanisms to salinity stress included; increased deposition of tannin in palisade parenchyma tissue, increased length of palisade cells and reduction in intercellular spaces (Figs.,4a, 5a, 6a ,7a&7b). Palisade parenchyma tissue tended to adopt a narrow elongated shape with the same size and arrangement towards both leaflet surfaces. The enlargement of mesophyll cells is evident. These cells are arranged without leaving intercellular spaces. Activity and high ratio of mesophyll area/ leaf area may cause increment in photosynthetic rate in xerophytes under favorable water supply conditions [20, 21].

The most noticeable differences between stressed and control *L. leucocephala* stems were the decrease of cambial activity in the vascular bundles in stressed plants and development of an interfasical cambium between collateral bundles(Figs.,4b,5b,6b,7c&7d).In this respect, Junghans *et al.,* (2006)[22] showed that high salt concentrations reduced the cambial activity in *Populous euphratica*. Cambial activity is very sensitive to different stresses. For example, in typical desert shrubs, drought stress led the period of cambial activity coincides with the rainy season [23].Also, formation cortical region by small chlorenchyma cells, homogeneous in shape and size, arranged in a compact way and containing abundant tannins. Increased deposition of tannin in epidermis layer and in pith by increasing salinity stress and the presence of druses in the collenchyma layer next to the epidermis. While the number of xylem row and number of vessels bundles increased by increasing salinity level up to 7500 ppm, these results are agreement with the finding of Abou-Leila *et al*.,(2012)[24].

The fiber tented to spread as a continuous layer between cortex and phloem in stem of stressed plants, and their numbers also increased with salinity. Canne-Hilliker and Kampny, (1990) [25] mentioned that the distribution of sclerenchyma tic tissue in the stem cortex and phloem is of considerable taxonomic value. While Yentür (2003) [26] indicated that sclerenchyma tissue provides an advantage against the loss of water.

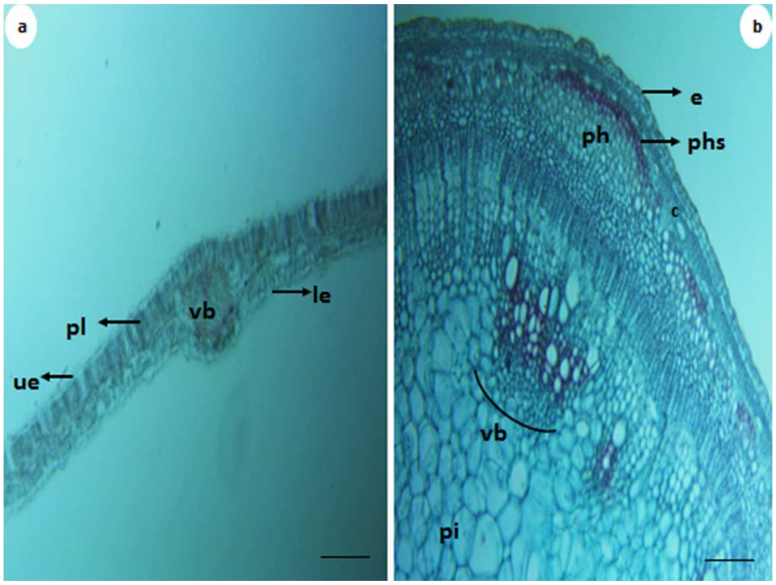

Figure 4. Cross-sections of *L. leucocephala* leaflet (a) and stem (b) irrigated with tap water (Bar= 200μm) ;e: epidermis , ue: upper epidermis ,le :lower epidermis, c: cortex, t: tannin cells, vb: vascular bundle, ph: phloem, phs: phloemsclerenchyma, pi: pith,

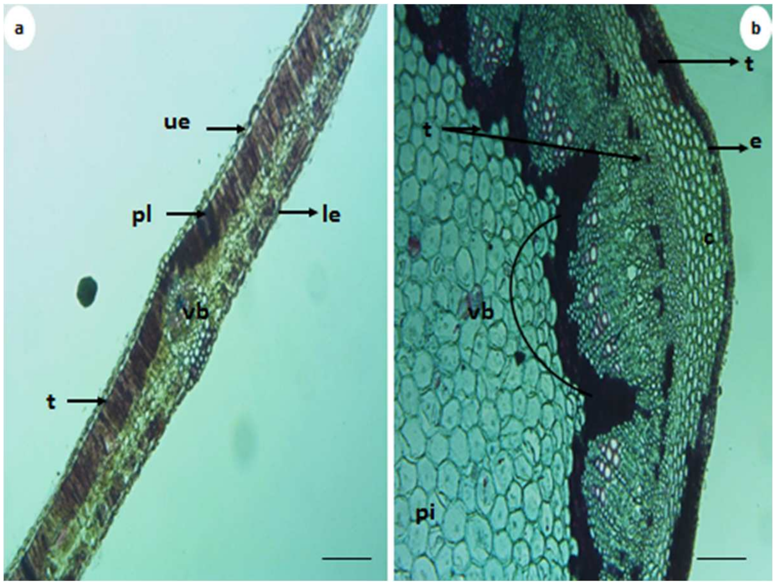

Figure 5. cross-sections of *L. leucocephala* leaflet (a) and stem (b) irrigated with 3500ppm saline water (Bar = 200μm);e: epidermis , ue: upper epidermis ,le :lower epidermis, c: cortex, tannin cells, vb: vascular bundle, ph: phloem, pi: pith.

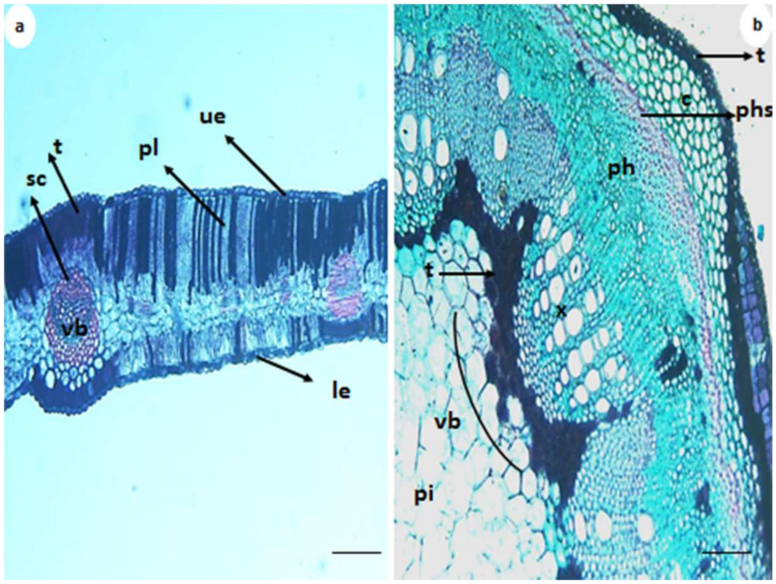

Figure 6. Cross-sections of *L. leucocephala* leaflet (a) and stem (b) irrigated with 7500ppm saline water(Bar = 200µm);e: epidermis, ue: upper epidermis ,le :lower epidermis,c: cortex, sc : sclerenchyma: tannin cells,vb: vascular bundle, pl: palisade parenchyma, ph:phloem, phs: phloemsclerenchyma, pi: pith,

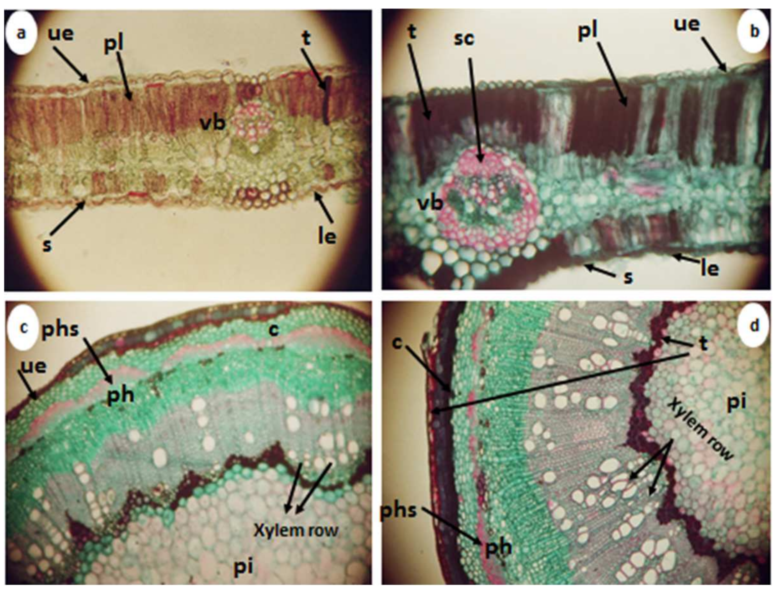

Figure 7. Cross-sections of *L.leucocephala* leaflet irrigated with 3500ppm saline water (200X) (a) and 7500 ppm(200X) (b)illustrate the elongation of palisade parenchyma, reduction in intercellular spaces and deposition of tannins in palisade cells ; cross-sections of *L. leucocephala* stem irrigated with 3500ppm saline water (100X) (c) and 7500 ppm (100X) (d) clarify the deposition of tannins in epidermis layer and pith and an increase in the number of xylem row and number of vessels bundles in Fig.d;e: epidermis, ue:upper epidermis, le: lower epidermis,c: cortex, sc :sclerenchyma, pl; palisadeparenchyma, s:stomata, t: tannin cells, vb: vascular bundle, phs: phloem sclerenchymapi: pith.

The cross sections of *Prosopis* leaflets irrigated with 3500 and 7500 ppm saline water (Figs., 8a, 9a &10b) showed funnel-shaped tannin-filled cells (arrow) located among the adaxial palisade tissue cells. *P.chilensis* responded to salinity by increasing the number of funnel shaped tannin cells and increasing the deposited tannin in the epidermis. In response to different stress conditions, the production of these UV-B absorbing phenolic compounds is considered to be a hallmark of adaptation to the terrestrial environment. The vascular bundles of both plants were collateral and surrounded by bundle sheath. Fibers were present external to the functional phloem of major veins.

In *Prosopis* stems, the deposition of tannin filled cells in the epidermis and in parenchyma cells of pith was increased by increasing in salinity stress (Figs.,8b, 9b &10b) .While the sclerenchyma fibers were connected in the interfascicular region , forming a continuous sclerenchyma cylinder. The vascular systems of *Prosopis* stems are also affected due to salinity, since vascular cylinders were very poor with less and narrow xylem vessels arranged radialy (Figs., 8b&9b). These results are agreement with those reported by others. Casenave *et al.,* (1999) [27] reported that with an increase in salinity there was a decrease in the development of the xylem. Pimmongkol *et al.,* (2002) [28] stated that the width of vascular bundles and diameters of rice stems decreased in NaCl medium. Similar results were also presented by Kutlu *et al*.,(2009)[29].

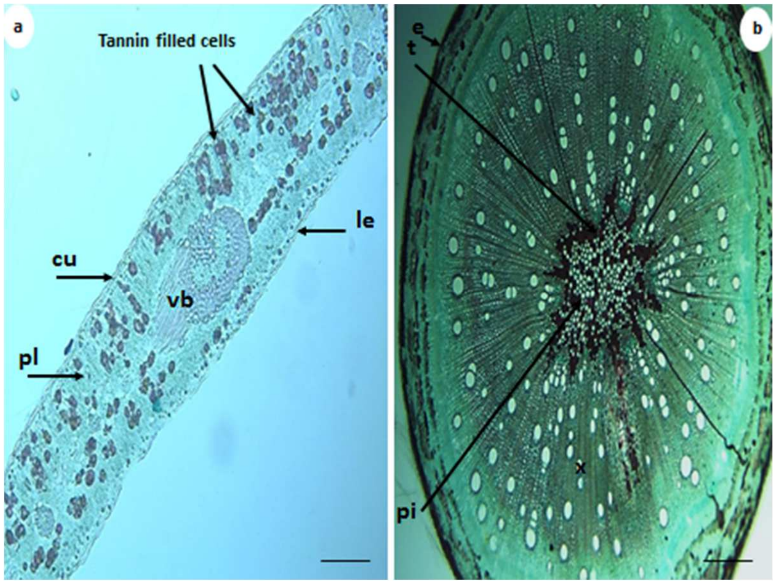

Figure 8. Cross-sections of *Prosopis* leaflet (a)(Bar = 200µm) and stem (b)irrigated with 3500saline water(Bar = 500µm);cu: cuticle, ep: epidermis, ue: upper epidermis ,le :lower epidermis, c: cortex, sc :sclerenchyma ,pl: palisade parenchyma, t: tannin cells,vb: vascular bundle,pi: pith.

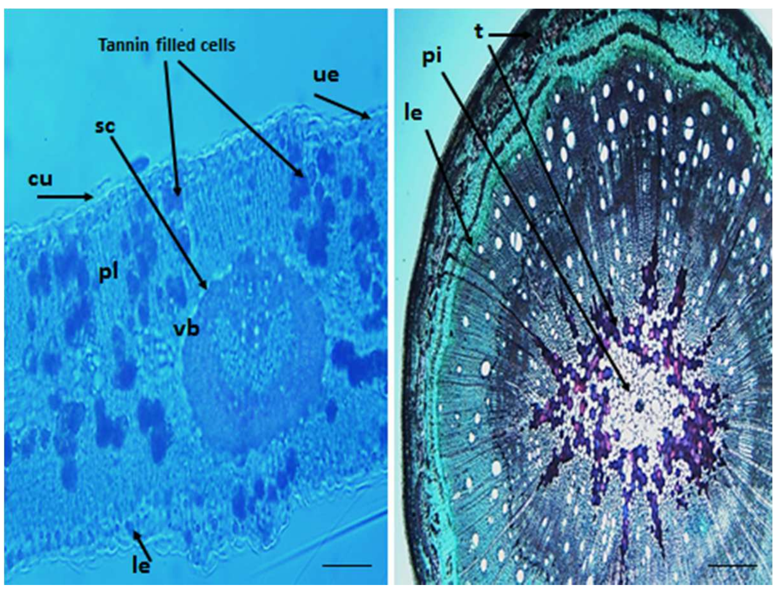

Figure 9. Cross-sections of *Prosopis* leaflet (a) (Bar = 100µm) and stem (b)irrigated with 7500saline water(Bar = 500µm);cu: cuticle, e: epidermis , ue: upper epidermis ,le : lower epidermis, c: cortex, sc :sclerenchyma , pl: palisade parenchyma, t: tannin cells vb: vascular bundle, pi: pith.

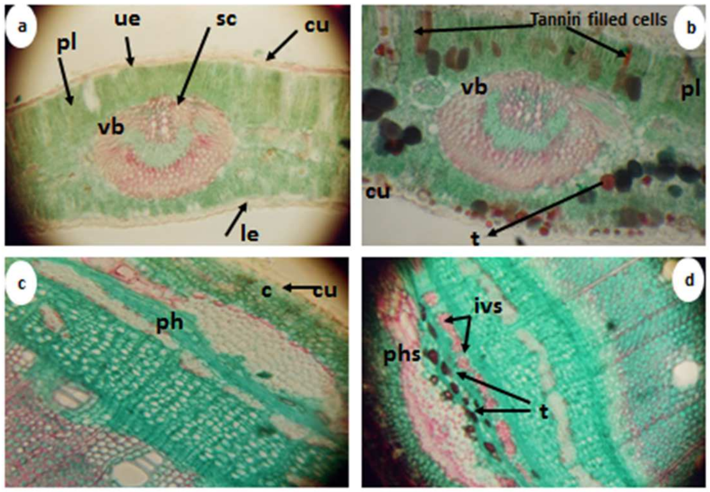

Figure 10. Cross-sections of *Prosopis* leaflet plants irrigated with tap water (200X)(a) and 7500 ppm saline water (200X)(b) illustrate the presence of funnel shaped cells filled with tannin (arrow)in Fig. band absence of these cells in control plant ; cross-sections of *Prosopis* stem plants irrigated with tap water (200X)(c) and 7500 ppm saline water (200X)(d) clarify the present of tannin –filled cells in the cortex and the presence of continuous sclerenchyma cylinder; cu: cuticle, e: epidermis, ue: upper epidermis ,le :lower epidermis, c: cortex, ph: phloem, phs: phloem sclerenchyma ivs: intravascular sclerenchyma, pi: pith, pl: palisade parenchyma, sc:sclerenchyma, t: tannin cells vb: vascular bundle.

GENERAL CONCLUSIONS

Plant tissues responses to water stress and salinity depend on the anatomic characteristics that regulate the transmission of water stress effect to the cells [30,31]

Comparing anatomical structure of the studied plants as control and salt stressed plants, it could be concluded that, there are some common morphological anatomical modifications of the investigated plants in response to salinity stress.

Irrigation with high level of salinity (7500 ppm) causing reduction in plant height of *Leucaena* and *Prosopis* in comparing with low level of salinity and delay in fruiting of *Prosopis*. Irrigation with the same concentration of salt water caused an increase in accumulation of tannins in stem and leafless of *Leucaena* and *Prosopis* plants. This is in agreement with the observation of a reduction in tannin content in palisade cell layers of *Kandelia candel* plants collected from a saline soil and grown in fresh water [32]. Studies by Fahn and Cutler (1992) [[23] led to the same conclusion, since accumulation of tannins was increased with drought in Australian sclerophyllous plants. These compounds may be involved in cell protective roles such as scavenging of reactive oxygen species, a secondary oxidative stress in abiotic and biotic stresses [33]. Therefore, results of the present investigation support that the accumulation of some phenolic compounds under stress can be considered as an adaptive response to condition under which the functions of these compounds become more important.

A waxy deposit on cuticle of both plants leaflets creates a rough shield against high irradiance. A waxy cuticle development is enhanced by arid environmental factors, thick cuticle increase epidermal reflectivity which in turn prevents a significant fraction of light entering the leaf [34,35]. The epidermal cell walls and cuticles of leaflets of salinized plants are thicker. The plant cuticle is a lipidic layer of cutin that covers essentially all aerial organs and functions to restrict transpiration. By this mechanism, the cuticle is thought to play a critical role in plant tolerance through its ability to postpone the onset of cellular dehydration during stress [36, 37].

Leucaena leucocephala and *Prosopis chilensis* plants showed a range of anatomical adaptive features like thick epidermis, formation cortical region by small chlorenchyma cells, homogeneous in shape and size, arranged in a compact way and containing abundant tannins .Reduction in intercellular spaces and elongation of palisade cell rich with many chloroplasts. Increased deposition of tannin in epidermis layer and in parenchyma cells of the pith by increasing salinity stress in both plants and accumulation of tannin-filled cells in the adaxial epidermis of leaflets in *P.chilensis*. All these modifications are capable of minimizing adverse effects of salt stress.

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