Photosynthetic and Leaf Anatomical Characteristics of the Drought-resistant *Balanites aegyptiaca* (L.) Del. Seedlings

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Abstract: Leaf anatomical characteristics of four months old *Balanites aegyptiaca* (L.) Del. seedlings and its relation to photosynthetic capacity were investigated. Scanning Electron Microscopy of simple, lanceolate elliptic to oval and divided true compound leaves showed larger simple leaf area and marginal leaf thickness, than in divided leaf. Adaxial surface of simple leaves exhibits less stomatal frequency ($48\pm3mm^{-2}$) than in abaxial surface (68 ± 4 mm^{-2}). Divided leaves, adaxial and abaxial surfaces showed almost similar stomatal frequency which ranged from 90 to $93\pm3mm^{-2}$. Stomatal pore length in both simple and divided leaves was 10.56 ± 0.5 μm on abaxial surface and 8.5μm±0.6 μm on adaxial surface. Unicellular trichomes frequency of both simple and divided leaves adaxial surface was $6\pm1mm^{-2}$. Highest trichome frequency ($13\pm4mm^{-2}$) was found on abaxial surface of divided leaves. Trichome length ranged from 500 to 538 ± 45 μm on both simple and divided leaves adaxial and abaxial surfaces. In general SEM revealed slightly rough or rough outer cuticle covering on the outer surface of the epidermis. Light microscopy of simple and divided leaves showed generally presence of intercellular spaces in mesophyll palisade parenchyma, reduction of divided leaf size in comparison with simple leaves. Palisade cells thickness varied from 304 to 155.5 μm in adaxial and abaxial surfaces of simple leaf, respectively and 177.7 μm in divide leaf adaxial and abaxial surfaces. Photosynthetic capacity of fully expanded leaves was measured in well watered *Balanites* seedlings under different Photosynthetic Photon Flux Density (PPFD) ranged from zero to 2000 μmol $m^{-2}s^{-1}$ at 35°C, light compensation points of P_n were 4.61 and 2066 μmol $m^{-2}s^{-1}$ respectively and CO_2 compensation and saturation points of C_i were 97.9 and 1744 μmol mol^{-1} respectively at 2000 μmol $m^{-2}s^{-1}$ (PPFD). *Balanites* simple leaf anatomy provides characters that are of great CO_2 assimilation and drought resistance during the first stage of seedling establishment.

Key words: Net photosynthetic flux density · photosynthetic photon flux density · intercellular CO_2 concentration · trichomes and stomata

INTRODUCTION

In the south eastern desert, high irradiation create a great challenge for establishment of endemic species plantation. *Balanites aegyptiaca* (L.) Del., is a slow growing, drought resistant and stress-tolerant evergreen, multipurpose tree species that grows within wide ecological amplitude through semi to extremely arid African areas [1-3]. Populations *of Balanites* as an indigenous desert tree existing in the upstream and middle regions of Wadi Allaqi, South Eastern Egyptian desert require protection and plantations establishment in the downstream region close to shores of Lake Nasser [4].

Previous research has been carried out in wet forest and little attention drawn to leaf anatomical characteristics correlated with CO_2 assimilation and drought resistance in extremely arid conditions, where diurnal seasonal PPFD maxima varied from 1900 to 2400 μmol $m^{-2}s^{-1}$ (Fig. 1). High irradiation in such arid environment may cause several changes in photosynthesis rate in many species [5].

Balanites water use efficiency tends to increase with elevated PPFD and slight decrement at high PPFD above 500 μmol $m^{-2}s^{-1}$ [6]. *Balanites* photosynthesis rate stability starts at 200 μmol $m^{-2}s^{-1}$ PPFD reaching the maximum at 2000 μmol $m^{-2}s^{-1}$ with no further increment [7]. The endemic *Linze Jujuba* shrub which grows in arid north west china, showed photosynthesis rate adapts to high temperature and radiation and high water use efficiency which is similar to that of desert plants [8].

Arid environments stimulate structural adaptations, which may take place in leaves, high ratio of mesophyll area/leaf area may cause increment in photosynthetic

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activity and transpiration rate in xerophytes under favorable water supply conditions [9-11]. Small but abundant stomata are also believed to lower resistance CO_2 diffusion to photosynthesizing mesophyll tissue, mesophyll compartmentalization could protect leaf against water stress, abundant palisade tissue also believed to increase the CO_2 absorbing surface of the mesophyll, Light reflectance and drought resistance features of many xerophytes species could be achieved by the abundance of trichomes on leaf adaxial and abaxial surfaces and presence of rough cuticle [11-14].

The objective of this study is to examine the leaf anatomical features of *Balanites* seedlings which correlates with photosynthesis and drought resistance under high irradiation conditions prevailing in extreme arid desert. Also, the leaf anatomical features of plant seedlings and their contribution to CO_2 assimilation rate under full range of PPFD is investigated.

MATERIALS AND METHODS

Plant material

Seed collection and germination: Fruits of *Balanites* were collected from Wadi Allaqi in 2004 and were soaked in boiled water overnight. Yellowish exocarp were scribed, the tips of hard stone seeds were mechanically appraised with sand paper and they were sowed in 25 cm diameter pots filled with garden soil 1:2 clay to sand. The pots were watered using distilled water, soil moisture content was maintained at field capacity of 8±1%.

Homogenous four months old seedlings were selected and kept in incubator at 30/25°C±1°C at 12:12 L:D cycle. High pressure sodium lamps were providing photoperiod of 400-700 μmol m^{-2}s^{-1} PPFD.

Carbon dioxide measurement: Net photosynthesis rate P_n and intercellular CO_2 concentration measurements were carried out using a LI-COR 6400 portable photosynthesis system LI-COR, Nebraska, USA. Inside the chamber, the programmed CO_2 concentration was 400 μmol m^{-2}s^{-1}. Light response curve was measured at PPFD ranged from 0 to 2000 μmol m^{-2}s^{-1} using LED type 6700 nm as artificial light supply.

Data analysis: Photosynthetic characteristics were obtained from light response curve P_n-*PPFD* at low points of light intensity PPFD=150 μmol m^{-2}s^{-1} and linear regression equation, light compensation (LCP) and

Apparent Quantum Yield (AQY) were calculated. The Light Saturation Point (LSP) was calculated at PPFD>150 μmol^{-1}m^{-2}s^{-1}. From linear regression analysis of P_n and C at low points= 50 μmol m^{-2}s^{-1}, CO_2 compensation point (CCP) and Carboxylation Efficiency (CE) were calculated, CE= dP$_n$/dC$_i$ [8].

The CO_2 saturation point (CSP) was calculated from P_n-C_i response curve under C_i>100 μmol m^{-2}s^{-1} [15].

Linear regression analysis was carried out using Minitab 12-21[16].

light and scanning microscopy

Material collection: Three fully expanded leaves simple and divided, were sampled at random from three different seedlings. All samples were fixed in 3% gluteraldehyde in phosphate buffer; and dehydrated in graded ethanol or n-butanol series [17].

Light microscopy: Samples were embedded in wax and sectioned at 10μm on a manual rotary microtome. Photographs of section were taken with Zeiss Ultraphot II, direct measurements of T.S. using stage micrometer and counting serial sections.

Scanning electron microscopy: Were further dehydrated in acetone series; dried with CO_2 at critical point and mounted with a double slide adhesive tape on stubs, sputter coated with gold and observed with a Hitachi S-520 S.E.M. Photographs of SEM images were taken with Nikon 35 ml camera [18].

RESULTS

Response of photosynthesis rate P_n to Photosynthetic Photon Flux Density (PPFD) and intercellular CO_2 concentration C_i P_n-*PPFD* response curve (Fig. 2) at low points of PPFD = 150 μmol m^{-2}s^{-1} Light Compensation Point (LCP) and Apparent Quantum Yield (AQY) were 4.61 μmol m^{-2}s^{-1} and 0.025 μmol mol^{-1} respectively. Dark and photorespiration point were 0.12 and 0.46 μmol m^{-2}s^{-1}, respectively. On the other hand, at high points of PPFD>150 μmol m^{-2}s^{-1} light saturation point LSP was 2066 μmol m^{-2}s^{-1}.

From P_n-*PPFD* response curve (Fig. 3) at C_i=100 μmol m^{-2}s^{-1} low points CO_2 compensation point (CCP) and carboxylation efficiency (CE) were 97.9 μmol mol^{-1} and 0.004 μmol mol^{-1}, respectively. While at C_i>100 μmol mol^{-1} high points CO_2 saturation point was 1744.9 μmol mol^{-1}.
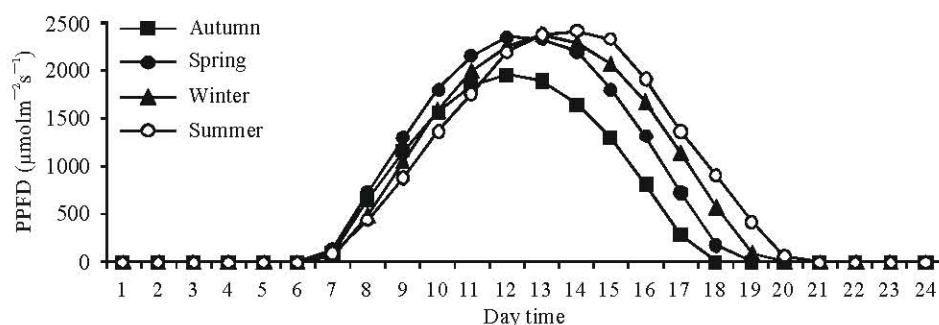
Fig. 1: Seasonal diurnal Phosynethtic Photon Flux Density (PPFD) average in Wadi Allaqi, South-Eastern desert, Egypt (2001-2006)
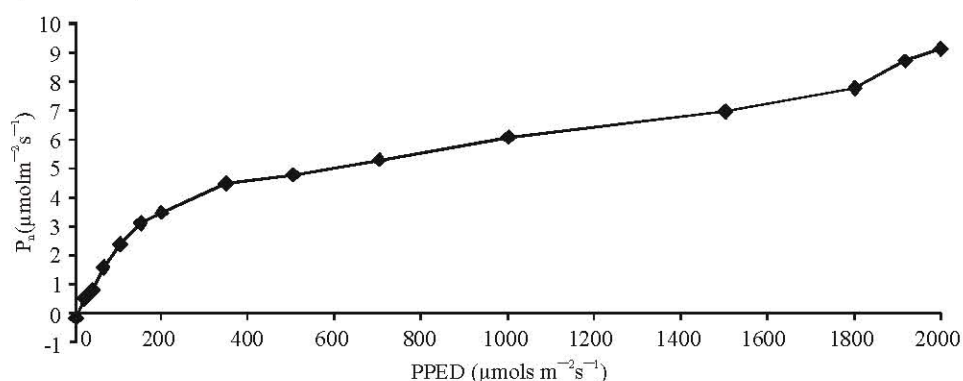

Fig. 2: Responses of net photosynthesis rate (Pn) to Photosynthetic Photon Flux Density in (PPFD) in *B. Aegyptiaca* leaves
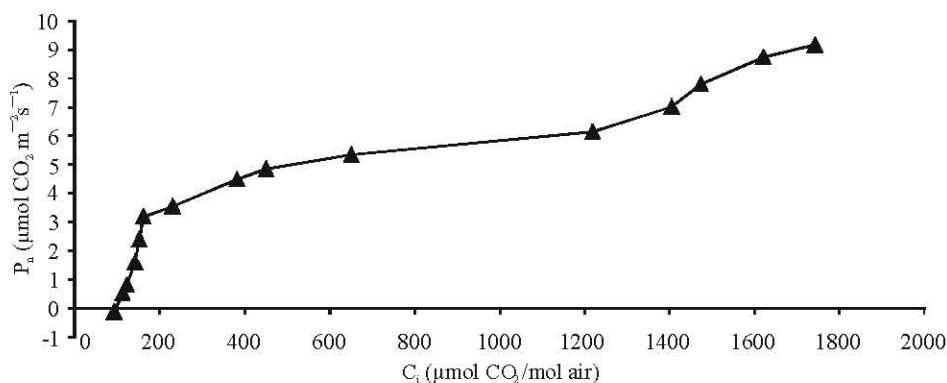

Fig. 3: Responses of net photosynthesis rate (Pn) to intercellular CO_2 concentration (Ci) in *B. aegyptiaca* leaves

Stoma guard cell and pore were elliptical (Plates 1B-E and 2B-E), 30-36 μm long and 27-30 μm wide and randomly distributed over the surface with no regular orientation. Stomata were present on both adaxial and abaxial surfaces of simple leaves and divided leaves and were sunken with obvious sub-stomatal cavities.

SEM of *Balanites* simple leaves showed that, stomatal density of abaxial surface (Plate 1A) was $68\pm4mm^{-2}$ compared to the adaxial surface with value $48\pm3mm^{-2}$ (Plate 1.D). On the other hand, divided leaf (Plate 2A and 2D), stomatal density ranged from 90 ± 7 to $93\pm7mm^{-2}$ on adaxial and abaxial surfaces, respectively.

Plate's 1.B-E and 2B-E revealed that, stomatal pore lengths of both simple and divided leaf adaxial are almost equal (8.5 ± 0.6 μm), while stomatal pore length of both simple and divided leaf abaxial surface was larger (10.65 ± 0.5 μm). The highest trichome density $13\pm4mm^{-2}$ was observed on divided leaf (Plate 2C) abaxial surface.
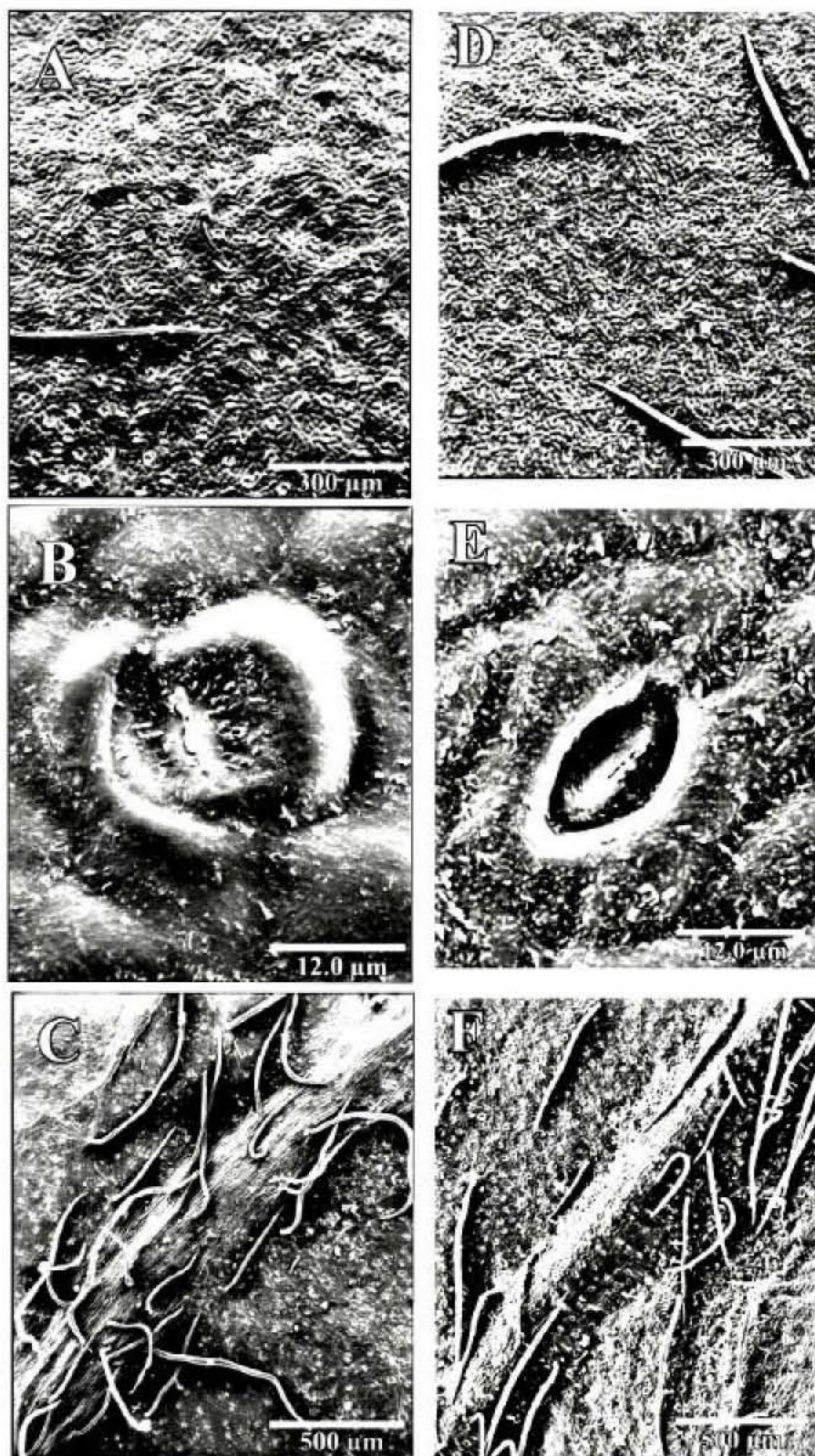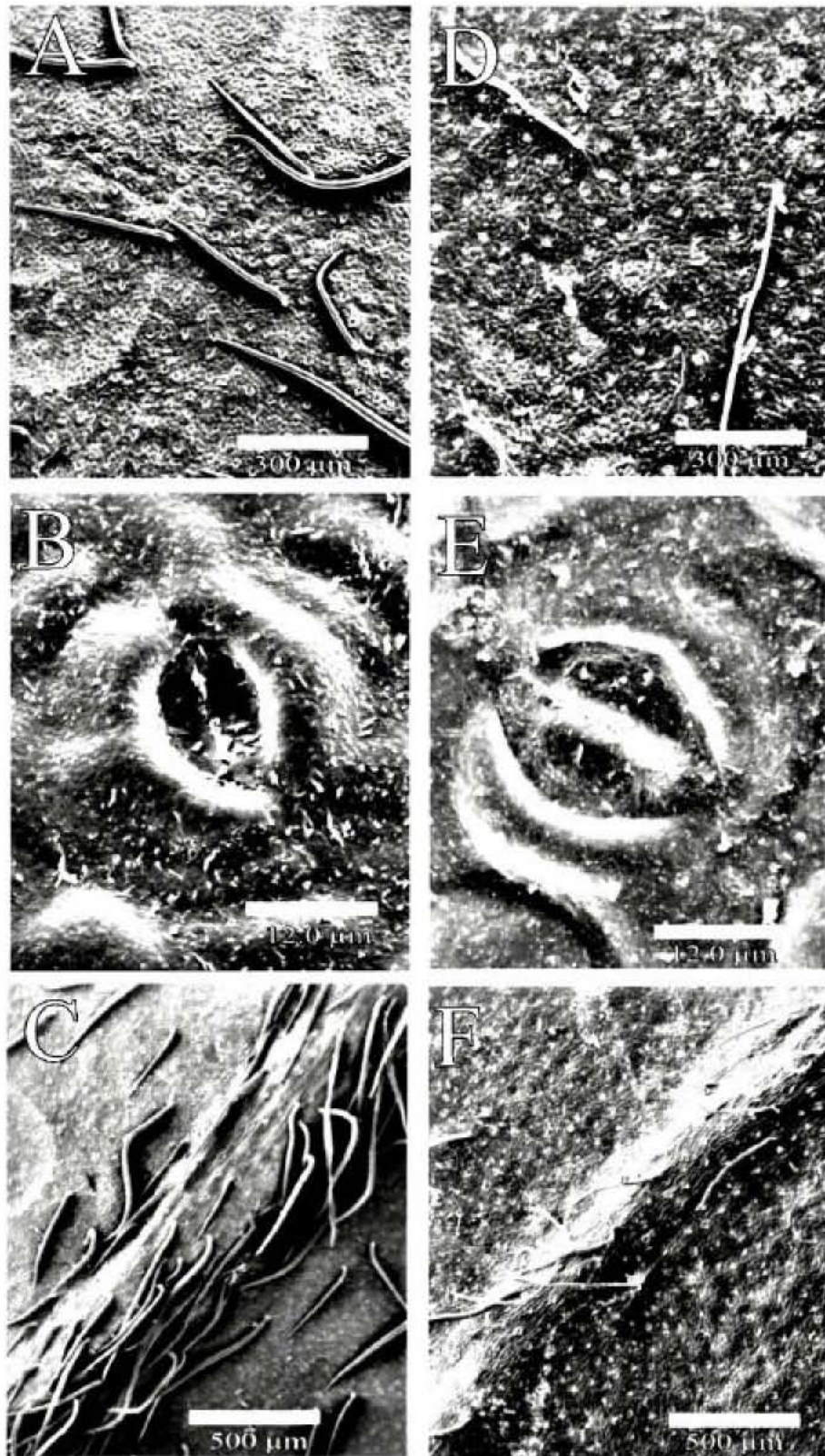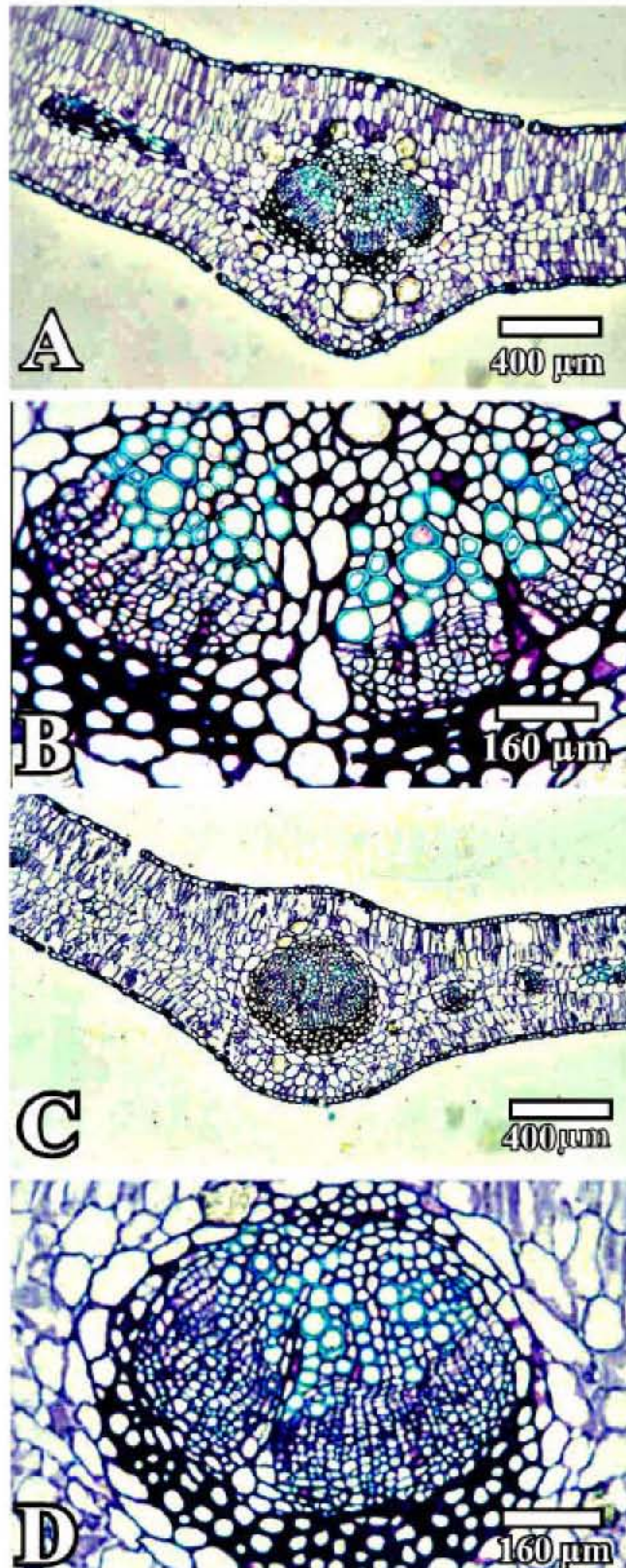
Plate 1:

Plate 2:

Plate 3:

In general, trichome density tends to increase on midrip area in both simple and divided leaves on adaxial and abaxial surfaces (Plate 1C-F and Plate 2C-F), while trichome length was ranged from 500 to 577±38μm.

Detailed light microscopy of simple leaf (Plate 3A-B) and divided leaf (Plate 3C-D) revealed that, both simple and divided leaves exhibited cuticle was approximately 8-16 μm thick and forming rough surface and presence of wax deposition on the outer walls. Below the upper epidermis and above lower epidermis, palisade was multi-layered, two to three layers representing almost two thirds of leaf thickness. Palisade parenchyma consisted of small, cylindrical cells tightly packed with presence of intercellular spaces. Below the palisade mesophyll was the spongy mesophyll layer.

The mesophyll cell contained numerous numbers of chloroplasts which were pressed against the cell walls and exposed to intercellular channels. There were two distinct, but similar layers of palisade mesophyll tissue arranged directly beneath the epidermis and flanking a central band of spongy mesophyll tissue. On the upper side of the lamina, the palisade consists of two to three layers; while on the lower side, the palisade layer was thinner two cells deep. Simple leaves showed larger palisade cells thickness 304.4±24μm and 155.5±12 μm in upper and lower leaf layers, respectively. While smaller palisade cells thickness was observed in divide leaf 177.7±9 and 111.1±6 μm in upper and lower leaf layers, respectively. The main vein was generally situated almost at the centre both horizontally and vertically. It comprised several lignified xylem vessels and large proportion of phloem tissue. Beneath and partially surrounding the phloem and xylem vessels were lignified sclerenchyma tissue, which was in turn enclosed with a bundle sheath of living parenchyma cells. Minor veins occurred along the entire lamina. The main vein complex in simple leaf (Plate 3B) was larger than that in divided leaf (Plate 3D). The main vein in simple leaf is characterized by presence of schizogenous gland beneath the vascular bundle.

DISCUSSION

Anatomical characteristics of *Balanites* simple leaf exhibits more thickness of laminae to receive enough light for photosynthesis during first few months of seedling growth. Reduced leaf size increased thickness, thick external walls of epidermal cells, high stomatal density and palisade developed at the expense of spongy mesophyll are common features of xeric environment plants [10, 19].

A relatively thick leaf as structural characteristics of xeromorphic leaves facilitate CO_2 uptake by mesophyll [14]. Abundant sunken stomata of *Blanites* on both adaxial and abaxial surfaces of simple and divided leaves, realize that decrement in CO_2 diffusion resistance may be attributed to stomatal abundance Lack of compartmentalization and cell arrangement with chloroplast distribution along cell wall facing intercellular spaces of palisade parenchyma [20]. A waxy deposit on cuticle of both surfaces adaxial and abaxial of Balanites leaves 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 [21-23]. The rough leaf surface of *Balanites* may increase turbulent air flow at the leaf surface, which promotes heat and vapor transport away from flow at the leaf surface [24]. Structural features of *Balanites* related to high solar radiation were small leathery leaves with equifacial thickening of the out epidermis and extremely thick cuticular layer on leaves and numerous stomata [25]. Although number of simple leaf is less than divided loped leaf, it represents more chances for CO_2 assimilation and establishment of *Balanites* seedlings.

Water stress decreases the size of leaves as a result of reduced cell expansion and cell division. On the hand, it leads to a reduction in proportion of epidermal cells that form stomata and increase number of epidermal trichomes [26]. One of the major anatomical features of *Balanites* leaves is the presence of trichomes on both leaf surfaces. The presence of leaf hairs tend to increase the reflectance of leaf surface, there is evidence that hairs on the lower surface of a leaf can increase reflection from the upper surface. Leaf hairs probably have greater significance for balance between water loss and assimilation. Many xeromorphic plants have an extreme development of structural tissues such as schlerenchyma and collenchyma as an implication for water conservation [27-29].

Photosynthesis is a physiological process that is affected mainly by the irradiance as one of the major environmental factors. In many desert species, drought tolerance is favored by increasing productivity through maximizing assimilation in relation to amount of available water [24]. Light response of net photosynthesis of desert plants compensation irradiance range was 20 to 350 μmol $m^{-2}s^{-1}$, while light saturation points>1500 μmol $m^{-2}s^{-1}$ [30]. On the other hand, light compensation point in

young seedling tend to be of lower values ranged from 2 to 5 $\mu mol\ m^{-2}s^{-1}$, for *Balanites* it was 4.61 $\mu mol\ m^{-2}s^{-1}$. Lower compensation point (LCP) and high saturation point (LSP) under apparent 400 ppm CO_2 concentration and quantum yield *Balanites aegyptiaca* could capture low light density and achieve greater photosynthetic capacity. Lower quantum yield high light intensity of 1300-1800 $\mu mol\ m^{-2}s^{-1}$ in young plants reaching 0.025 $\mu mol\ m^{-2}s^{-1}$ [31]. At low light intensity, dark respiration was 0.12 $\mu mol\ m^{-2}s^{-1}$, dark respiration plays an important role in production of required energy for plant growth [32].

High irradiance can suppress photosynthesis of young *Balanites* seedlings. The results of this experiment revealed that, at C_i=100 $\mu mol\ m^{-2}s^{-1}$ (CCP) CO_2 compensation point was high (97.9 $\mu mol\ m^{-2}s^{-1}$) and carboxylation efficiency 0.004 $\mu mol\ m^{-2}s^{-1}$ while (CSP) CO_2 saturation point at C_i=100 is 1744.9 $\mu mol\ m^{-2}s^{-1}$. C_i in the leaf intercellular spaces in this experiment, *Balanites* was found to have high CO_2 saturation point (CSP) at optimum photosynthesis rate P_n which reveals its high carboxylation efficiency at high PPFD (2000 $\mu mol\ m^{-2}s^{-1}$). The apparent quantum yield of (0.025 $\mu mol\ m^{-2}s^{-1}$) revealed that *Balanites* seedling leaves have more ability to absorb light quanta (mol photons) rather than photochemical work (mol O_2). This is considered as an adaptation for increasing the photosynthetic apparatus efficiency during development under high light stress conditions [30].

Other studies [30, 33, 34] revealed that, C_3 plants with laminar mesophyll and parenchymatic bundle sheath leaf structure exhibit the following photosynthetic characteristics: photosynthesis depression by Oxygen, CO_2 release in light (apparent photorespiration), slight to high photosynthetic capacity, light saturation of photosynthesis accurate intermediate intensities, slow redistribution of assimilation products and medium dry matter production.

The simple leaf anatomical characteristics proved that thicker leaf laminae, multilayered palisade parenchyma, presence of intercellular space of palisade parenchyma, compartmentalization of mesophyll more palisade create better opportunity for growth during seedling establishment in severe arid conditions.

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