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The anatomical features of the desert halophytes *Zygophyllum album* L.F. and *Nitraria retusa* (Forssk.) Asch

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Abstract The morpho-anatomical features of *Zygophyllum album* L.F. and *Nitraria retusa* growing on the El sokhna region, adjacent western coast of Red sea, Egypt, which contribute for their adaptation to the stressful habitat conditions were studied. An analysis of the morphological and anatomical characters of their leaves, stems and roots revealed representative of the halo-xerophilic characters among these plants. Adaptation is achieved by succulence, the presence of trichomes, sunken stomata, cylindrical leaves, laminate unifacial leaves, xylem fibers, sclerenchyma tissue, storage materials and crystals. The above mentioned morpho-anatomical features in response to the stressful conditions could be considered as anatomical function to adaptation to drought, salinity and heat stress.

Introduction

Studies on the halophilic vegetation of arid and humid areas have been attracted great attention (Pyankov et al., 2001; Khan and Weber, 2006).

Plants inhabiting in coastal areas have developed various mechanism and certain features providing not only for normal life functions under these conditions but also for adaptation to stress. These mechanism and features help them to thrive under adverse conditions and are often displayed in the morpho-anatomical changes in plants (Grigore and Toma, 2007; Hameed et al., 2009; Ashraf and Harris, 2013). *Zygophyllum album* and *Nitraria retusa* are habiting in coastal region of El Sokhna Suez governorate, Egypt (Kassas and Zahran, 1967).

Z. album (Zygophyllaceae) is perennial herb, branched stems with opposite bifoliolate succulent leaves. *Z. album* is one of the medicinal plants (Meng et al., 2002).

N. retusa (Nitrariaceae) is a thorny shrub 1–2 m with many stems, and leaves are simple, alternate, petiolate, fleshy, retuse or crenate-dentate at the apex: stipules minute. *Nitraria* is an indicator of shallow water table, sand controller, palatable to grazing animals, phytoremediator of polluted soil, and used as fuel and its fruits are edible by birds and local inhabitants (Shaltout, 2003), and medicinal plant (Louhaichi et al., 2011).

Anatomical structures of plant organs, especially leaves modified change thus enabling plant adaptation to its

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environment. Many leaf features have been recognized to provide a protection against various environmental conditions and stresses including drought, high air temperature and high concentration of salt in soil. Most morphological and anatomical adaptations of desert plant under saline habitats are smaller leaves, fewer stomata per unit leaf area, increased succulence, thickness of leaf cuticle and deposition of wax (Mass and Nieman, 1978).

The objectives of present study were to investigate morpho-anatomical features of *Z. album* and *N. retusa*, naturally growing under desert-saline habitats and to verify modified plant organs which encounter harsh conditions.

Material and methods

The studied plants *Z. album* and *N. retusa* were collected from Ain El Sokhna coast, Suiz Gulf 120 km. east of Cairo during the period of years 2011–2012. Specimens of the collected plants were pressed, dried, mounted as herbarial samples, and kindly identified by the staff of herbaria of Flora and Phytotaxonomy Researches Dept., Dokki, Giza. After cleaning the plants from the fine sand debris, representative parts of suitable size from all organs of each species (roots, stems, and leaves) at various growth stages were prepared. The Samples were fixed in FAA (formalin, acetic acid and 70% ethyl alcohol, 5:5:90/100 ml) for 24 h at room temperature. Then it dehydrated and processed using the schedule of the paraffin method according to Johansen (1940). Transverse sections (10–12 μm) in thickness were made by LEICA rotary microtome model RM 2125 RTS. Sections were stained with safranin-fast green combination (Johansen, 1940). Anatomical examination and measurements were achieved using a Leica light Research Microscope model DM-2500 supplied with a digital camera.

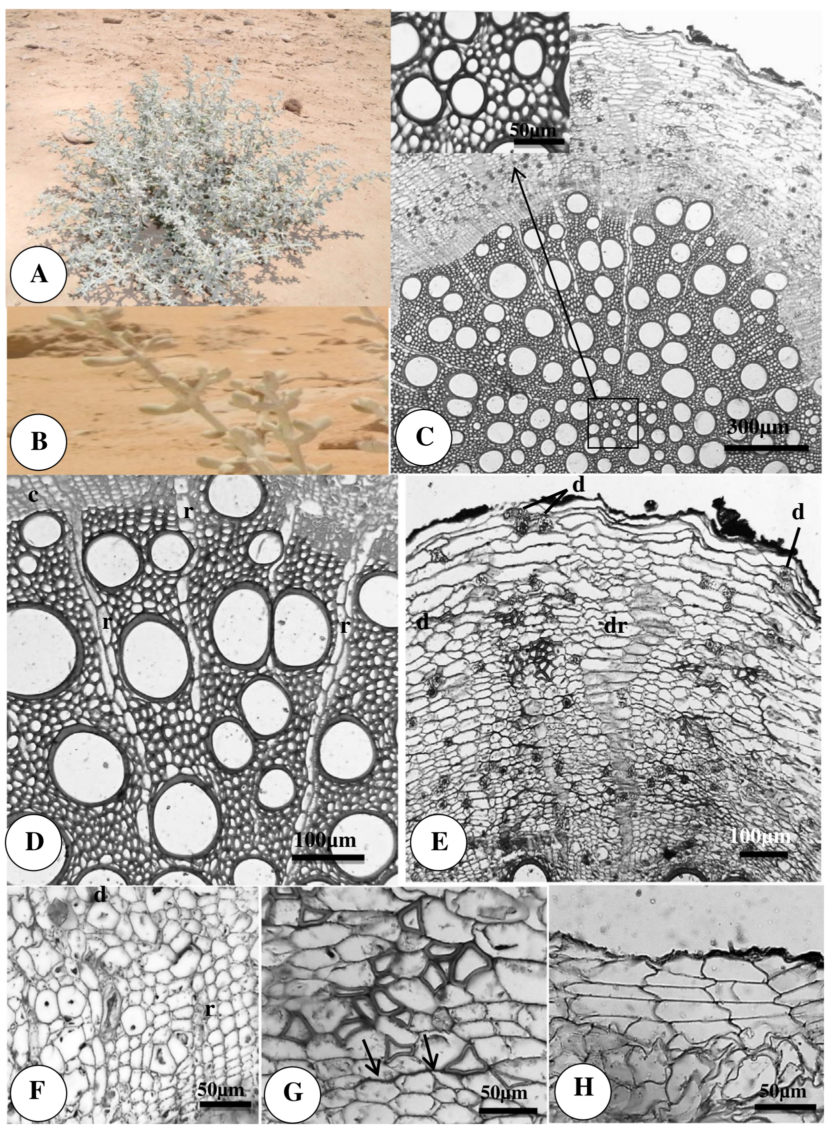

Fig. 1 **A**, **B**. Morphology of *Zygophyllum album* plant. **A**. General view. **B**. An enlarged part. **C**. Transection in an old root reveals its structure, and the upper box is a magnification of the root center illustrating the primary xylem. **D**. The structure of secondary xylem, note the uni- and biseriate rays. **E**. The outer part of the root. Note the dark spots point to druses. **F**. Functioning phloem. **G**. Fibers and the obliterated sieve elements (arrows). **H**. The layers of periderm. **d**, druse. **dr**, dilated ray.

Results

Zygophyllum album L.F.

The halophyte *Z. album* is a perennial herb (25–50 cm) gray in color with many succulent branched stems. The leaves are petiolate, bifoliolate and opposite. The petiole and the leaflet are cylindrical or obovoid. The petiole length ranged from 0.6 to 1.2 cm whereas that of the leaflet reaches 3 to 8 mm (Fig. 1A and B).

The root

The old root of *Z. album* consists of three zones: the periderm, the pericyclic derivatives and the vascular tissues (Fig. 1C). The primary xylem occurs in the center of the root since the later has no pith. It consists of two protoxylem poles (Fig. 1C). The secondary xylem consists of numerous vessels distributed solitary, or rarely in pairs. The diameter of most vessels ranges between 130 and 250 μm. Also, there are several narrow ones range between 27 and 91 μm in diameter. The vessels are embedded in lignified cells. Uni-or biseriate rays have been observed intervening the secondary xylem (Fig. 1C and D) and the secondary phloem (Fig. 1C). The phloem consists of sieve tubes with companion cells embedded in thin-walled parenchyma (Fig. 1F). There are few druses observed in the parenchyma of functioning or nonfunctioning phloem (Fig. 1F and G). Several lignified cells were observed in the nonfunctioning phloem either solitary or in groups of 2–8 cells (Fig. 1F and G). The vascular cambium and its derivatives occurred between the secondary phloem and the secondary xylem (Fig. 1E). The outermost layer of the pericyclic derivatives initiates the phellogen, which in turn, gives rise to the periderm (Fig. 1E and H). The latter consists of phellogen, an outwardly 7–9 layer with suberized wall of phellem and inwardly one cell layered phelloderm (Fig. 1H).

The stem

The young stem is composed of a uniseriate epidermis, a vascular cylinder and a ground tissue differentiated into a wide cortex and a small pith (Fig. 2A). The epidermal cells have a nearly isodiametric shape or slightly elongated tangentially with thick cuticle layer and provided with many unicellular trichomes and several slightly sunken stomata (Fig. 2B). The vascular cylinder consists of about ten collateral vascular bundles with different sizes. The interfascicular zones are very narrow (Fig. 2A). The cortex is parenchymatous. Groups of fibers were observed near the phloem intervening the cortical cells (Fig. 2A and C). The outermost layers of the cortex are differentiated into photosynthetic layer. Groups of small cells having one druse in each were shown in the cortex especially in the inner part near the phloem. One to seven cells in each group were observed (Fig. 2D). The pith on the other hand is parenchymatouse without chloroplasts. The cell groups with druses are also scattered in the pith.

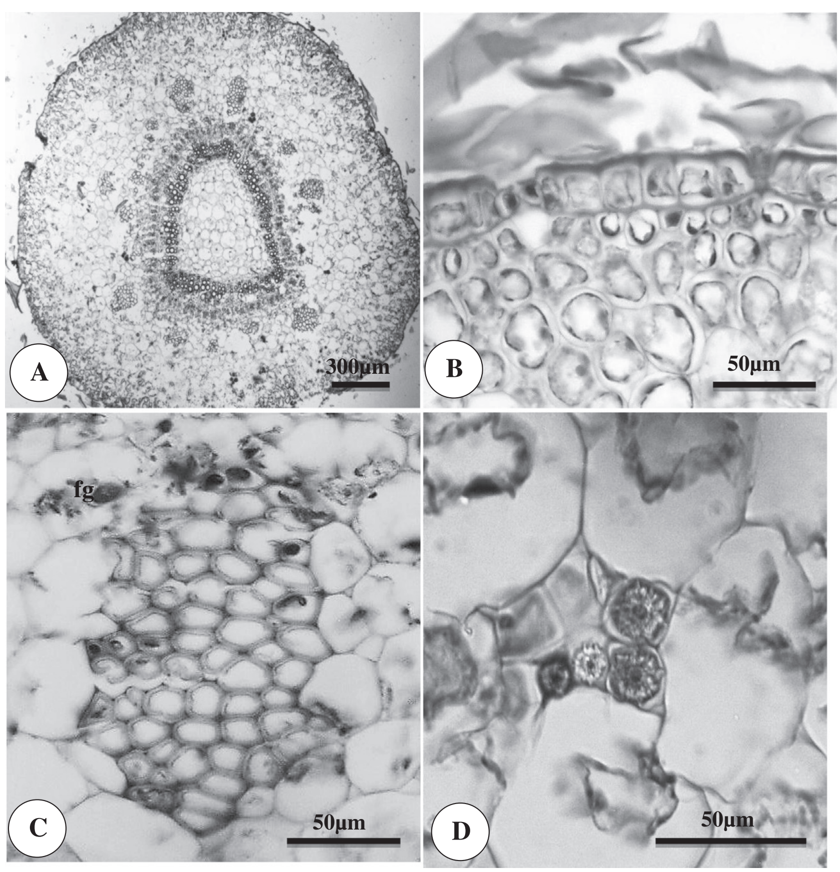

Fig. 2 **A**. Transection in a young stem reveals its structure. **B**. Reveals the epidermal cells, sunken stomata, trichomes and the thick cuticle. **C**. The fiber groups in cortex. **D**. The groups of druses.

The leaflet

Examination of leaflet cross sections reveals that epidermis is uniseriate layer enclosing a centric mesophyll. The latter is differentiated into photosynthetic tissue outward and water storage parenchyma inward (Fig. 3A). Mostly, the epidermal cells have an isodiametric shape with somewhat sunken stomata and numerous unicellular, biforked trichomes (Fig. 3B). The photosynthetic tissue of the leaflet consists of palisade cells arranged into 4–6 intervening layers. Their dimensions are 198 μm in length and 52 μm in width. A lot of idioblasts with druses were observed intervening the palisade tissue (Fig. 3C). The center of the leaflet is occupied with water storage tissue. It consists of 5–7 rows of wide, thin-walled cells. It forms about 55% of the leaf cross section area (Fig. 3A). The vascular tissue of the leaflet is differentiated into main central bundle and two small lateral ones embedded in the water storage tissue as well as numerous smaller bundles occur on the inner periphery of the palisade tissue or inserted in the innermost palisade layer (Fig. 3A and C). The peripheral vascular bundles consist of phloem toward the leaf center and xylem occurs outwardly. The phloem consists of sieve elements with companion cells embedded in phloem parenchyma. No phloem fibers were observed. The xylem consists of few narrow tracheary elements arranged into short rows with 2–4 elements in the midvein (Figs. 3C and D).

Nitraria retusa (Forssk.) Asch

N. retusa is a perennial shrub 1–2 m in length: mound-shaped. Stems have many spines. The leaves are simple, alternate, petiolate, obovate, fleshy, spatulate, entire or ending in 3–5 teeth (Fig. 4A and B).

The root

The old root of *N. retusa* is composed of three common zones: the periderm, the pricyclic derivatives and the vascular tissues (Fig. 4C). The primary xylem occurs in the center of the root and consists of two protoxylem poles (Fig. 4D). The secondary xylem consists of numerous vessels arranged solitary or rarely into pairs (Fig. 5C and E). The diameter of most vessels ranges between 30 and 91 μm. Also there are several narrow ones (9.5–22 μm in diameter). The vessels are embedded in lignified cells. Uni-, biseriate or triseriate rays have been observed intervening the secondary xylem and the phloem (Fig. 4E). The phloem showed sieve elements with companion cells embedded in thin-walled parenchyma. There are groups of parenchymatous cells stored starch at phloem zone (Fig. 4F). With increase in root girth due to secondary growth the primary phloem and the outer parts of the secondary phloem are being obliterated (Fig. 4F). Near the outermost layers of the secondary phloem there is ring of fibers surrounding the vascular tissues with two cells in width (Fig. 4C, E, and F). The outermost layers of the pericyclic derivatives initiate the phellogen, which give rises to the periderm. The latter consists of wide phellem layers outward and one-layered phelloderm inward. The phellem is composed of 6–15 rows of cells having an isodiametric shape or elongated one (Fig. 4G).

The stem

In transections the young stem (Fig. 5A) has a uniseriate epidermis in nearly isodiametric shape or slightly radially

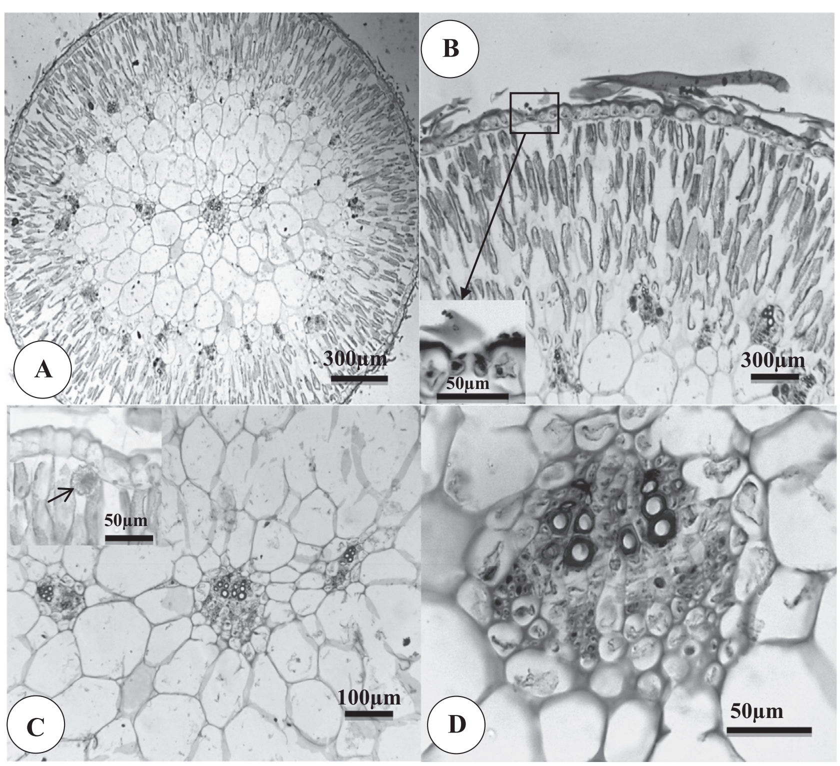

Fig. 3 **A**. Transverse sections reveal structure of *Z. album* leaf. **B**. Transverse section shows the epidermis, trichomes, mesophyll and enlarged view to stomata. **C**. The central part of leaf comprising the main central bundle and two of lateral ones, the druses in the mesophyll tissue (arrow). **D**. The structure of main central bundle.

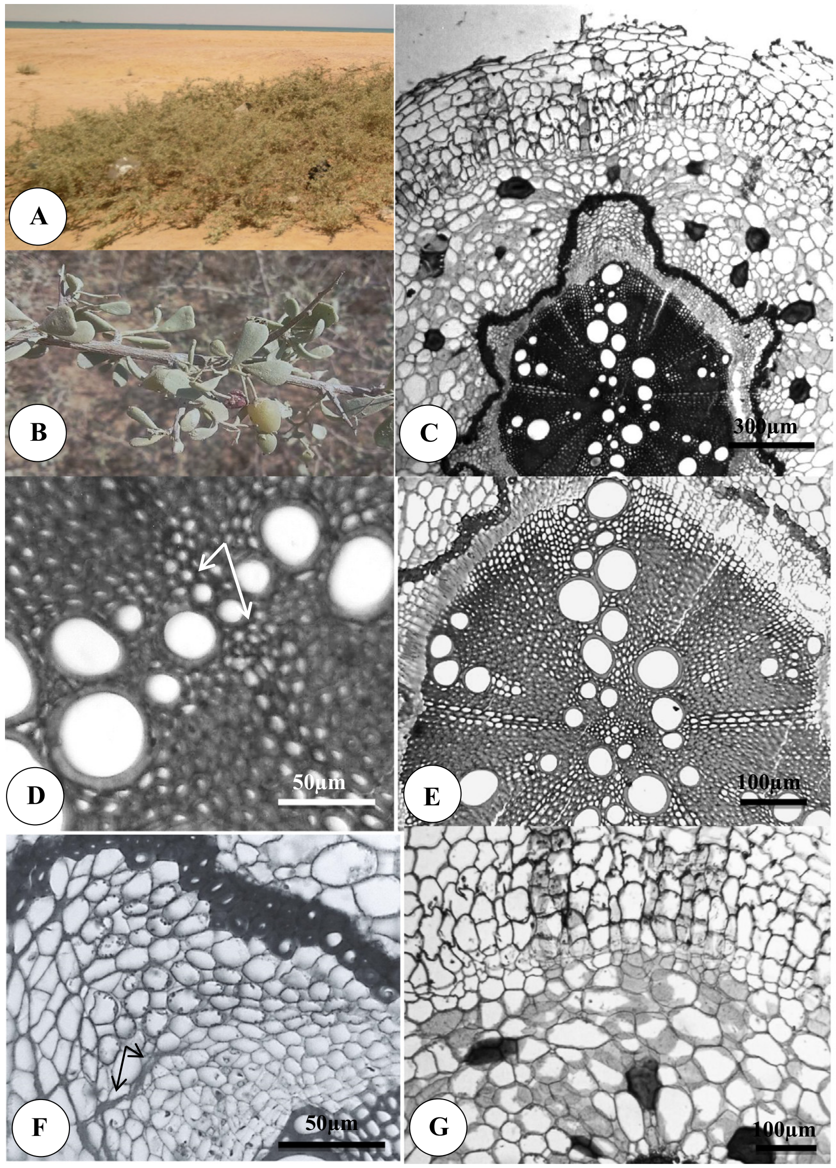

Fig. 4 A, B. Morphology of *Nitraria retusa* plant. **A**. General view. **B**. An enlarged part. **C**. Transection in an old root reveals its structure. **D**. The central part of the root, note the primary xylem (arrows). **E**. The structure of secondary xylem. **F**. Structure of secondary phloem (arrows reveal the obliterated phloem). **G**. The outer part of the root reveals the pericyclic derivatives and periderm reported the well-developed.

elongated cells. The epidermis provided with unicellular trichomes and few somewhat sunken stomata (Fig. 5B). The outermost cortical cell layers have many chloroplasts. There is not any kind of supported tissue observed in the cortex. The cortex is rich in dark cells (Fig. 5A and C). The innermost cortical layer is differentiated into starch sheath (Fig. 5C and D). The vascular cylinder consists of several collateral vascular bundles (Fig. 5A). The interfascicular regions are parenchymatous cells. The pith is parenchymatous and free of dark cells as compared to cortex cells.

The leaf

The leaf consists of upper and lower of simple epidermis enclosing in between the mesophyll. The latter is intervened by small vascular bundles (Fig. 6A). Both of epidermal layers are uniseriate with isodiametric cells in transections and covered with thick cuticle (Fig. 6A). Unicellular and unbranched trichomes were observed on both upper and lower epidermis. Stomata are Sunken and scattered on both upper (adaxial) and lower (abxial) epidermis. Then, the leaf is amphistomatic. The mesophyll tissue is differentiated into 3–5 irregular rows of palisade tissue toward both surfaces and spongy tissue in between (Fig. 6A). Then the leaf is unifacial. The dimensions of palisade cells are 97 µm in length and 41 µm in width. The spongy tissue consists of three layers. These cells are isodiametric, spherical, prolonged or irregular in shape (Fig. 6A and B). The mesophyll includes many idioplasts with dark contents and others with light contents. Most of these idioplasts are

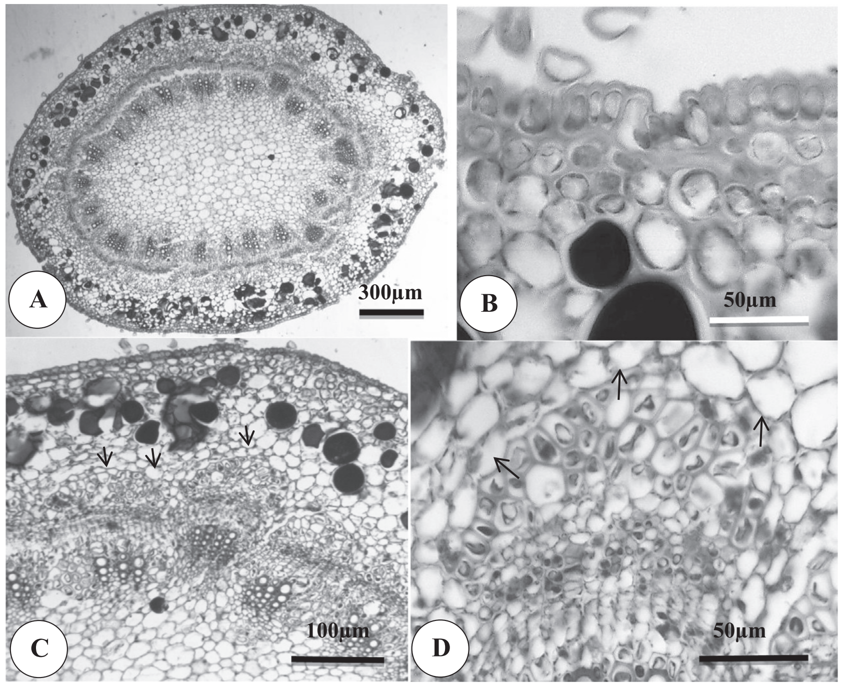

Fig. 5 **A**. Transection in a young stem of *N. retusa* reveals its structure. **B**. The epidermal cells with the thick cuticle and sunken stomata. **C**. An enlarged view reveals the cortex and the structure of the vascular bundles. **D**. Structure of the phloem. Note the starch sheath in **C**, **D** (arrows).

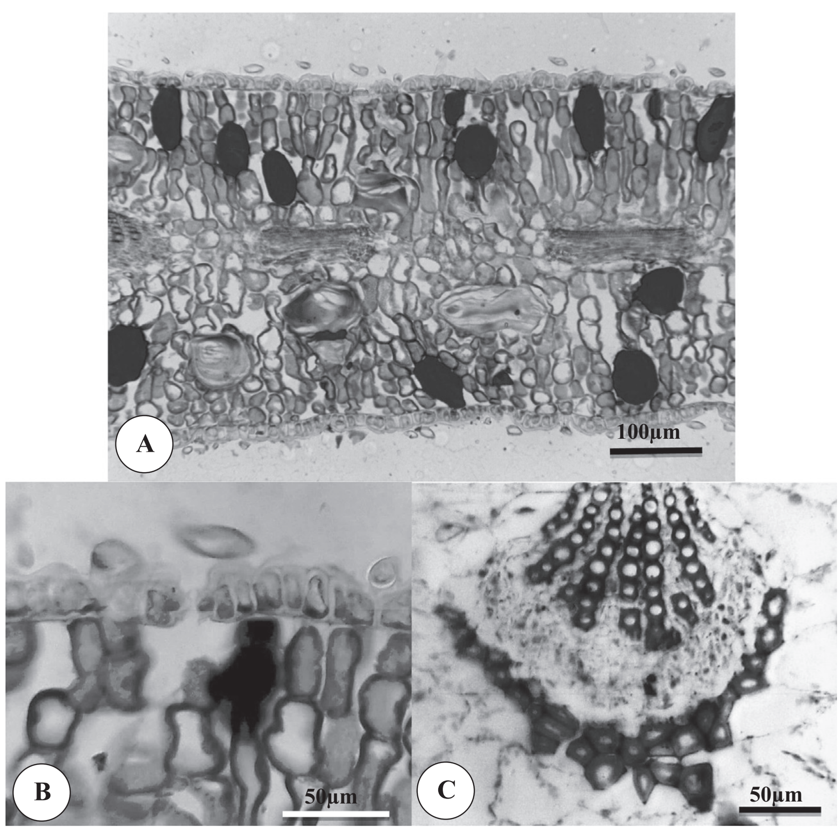

Fig. 6 **A**. The structure of the leaf. Note the mesophyll tissue and the dark cells. **B**. The epidermal cells, sunken stomata and the thick cuticle. **C**. An enlarged view reveals the structure of the midvein bundle.

adjacent to the epidermal layers (Fig. 6A). The venation of the leaf is reticulate. All the veins are embedded in the uppermost spongy layers. The midvein has large bundle consists of nearly eight or nine rows of tracheary elements. Each row has two or three xylem elements (Fig. 6C). The phloem occupies the lower side of leaf. It consists of sieve elements with companion cell, phloem parenchyma and phloem fibers (Fig. 6C).

Discussion

The plants inhabiting saline environments have developed certain features which help them to thrive under adverse conditions. Such features are often displayed in the morpho-anatomical and physiological changes of plants (Hameed et al., 2009; Ashraf and Harris, 2013).

Both of *Zygophyllum* and *Nitraria* were collected from Red sea coastal habitats and they are grown under exposed soil salinity, heating and strong winds in open coastal habitats may well provide for the development of halophytic and xerophytic characters in studied plants.

Our observations indicate that the secondary xylem vessels of the old root of *Zygophyllum* and *Nitraria* are embedding in lignified cells (fibers); perhaps, this is a mechanism that helps protection of water columns from embolism. This result was confirmed by Grigore and Toma (2007) who explained that. Lignin might confer resistance to the cell walls. This resistance is also involved in supporting and counteracting the high osmotic pressure which the halophytes have to face it in rhizosphere. This makes the root the most important interface between the plant and the hypersaline medium. Also, occurrence of lignified cells in high percentage of the old root may be an important for providing rigidity to these organs. Jacobsen et al. (2005) found that the presence of fibers around vessels contributes to cavitation resistance.

On the other hand, the phellem, i.e., the external part of the root could delay the water absorption. Thus, the salts penetrate hardly through the root, but once they get there they spread within this enlarged surface. The water distribution to the rest of the plant might literally ‘‘be delayed’’. The enlargement of the root surface confers a larger dispersion place for salts where they are diluted and finally less harmful to the plant (Grigore and Toma, 2007).

Generally, many xerophytes have thick cuticles and thick outer periclinal epidermal cell walls (Esau, 1977; Fahn and Cutler, 1992). The presence of thick cuticle layer covers the epidermis of stems and leaves of *Zygophyllum* and *Nitraria* seem to be fully justified by the better adaptation to adverse moisture limited conditions. The plants with waxy cuticle in the leaves and stems minimize the loss of water under salinity and drought stress conditions. Moreover sunken stomata accompanied with occurrence of high density of trichomes arising from the epidermis is adapting mechanism to minimize water loss under stressful habitat. These results agree with many authors who reported that the common traits of desert shrub leaves are small surface area, well developed epidermal hairs covering the leaf surface, stronger cutinization and sunken stomata, thus reducing water loss (Liu et al., 1987; Wang et al., 1991; Yan et al., 2002).

Z. album leaves showed cylindrical shape, and this shape tends to minimize heating caused by intense radiation of the leaves while *N. retusa* leaves have laminates shape, but accompanied an isolateral leaf anatomy. Isolateral (unifacial) leaf anatomy is common in plants growing under hot and arid environments because it tends to minimize heating of leaves and thus reduces transpiration demands (Ehleringer and Forseth, 1980). The succulence is another major strategy of halophytes.

The succulence is represented by a water storage parenchyma tissue formed by very large cells with relatively thin walls and channels among them.

Succulence exercises a dilution effect upon the salts accumulated in plants and upon the toxic ions from the cells, thus permitting the plant to cope with higher salt amounts (Waisel, 1972). The xero-halophytes are additionally characterized by a highly developed lignified vascular system (the case of *Z. album*). Therefore the ecological significance of succulence may be correlated with the local environmental factors. Thus, *Z. album* has been harvested from dry saline soils; it might be classified as a xero-halophyte which could explain the necessary presence of some water storage tissue (Grigore and Toma, 2007). The presence of succulence and the above-discussed dilution effect contributes to maintaining cellular turgescence that is also responsible for plant’s erected stature. This should be closely correlated with the rudimentary and weakly developed mechanical tissues of *Z. album*.

The present study indicated that there are many starch granules accumulated at the phloem parenchyma of the woody root in *Nitraria*. It could play an important role in osmotic adjustment in the salt-tolerant plants. This finding accommodated with the results of Ashraf and Tufail (1995), Murakeozy et al. (2003), and Abd Elbar et al. (2012)). Occurrence of fiber groups near the phloem in the stem of *Zygophyllum* may be an important as it would provide rigidity to the stem. This result matched with results of Zhang et al. (2003) and Abd Elbar (2015) where mechanical tissue and sclerenchyma around the vascular cylinder provide a good support and avoid drought. In the same context Huang et al. (1997) reported that Sclerenchyma in stems of *Ceratoides lateens* and *Peganum harmala* is useful for phloem to avoid damage from high temperature, intense radiation and drought.

The cortical and pith parenchyma of *Nitraria* stem is rich with dark cells. Their contents are tannins and mucilage according to Metcalfe and Chalk (1950) and Sheahan (2011). Also, the same cell appearance was recorded in the mesophyll of *Nitraria* leaves. Mucilage cells or jelly in many species can increase osmotic pressure, which improve water retention capacity and water absorption, providing a relatively wet micro-environment for surrounding photosynthetic cells (Jiang, 2004; Su et al., 2005).

Our results showed increase in the number of druses (calcium oxalate crystals) in *Z. album* organs. It can be assumed that calcium ions are involved in increasing the salt tolerance in different ways. At the same time our results indicate that the palisade layers occurred on both the upper and lower epidermis in *Nitraria* leaves agreeing with Zaman and Padmesh (2009) where the palisade in *N. retusa* leaves is arranged next to the upper and lower epidermis, while the spongy parenchyma is limited to the central location. This feature is typical of xerophytic adaptation.

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