Volume 62(1):25-36, 2018
Acta Biologica Szegediensis
http://abs.bibl.u-szeged.hu/index.php/abs
DOI:10.14232/abs.2018.1.25-36

ARTICLE

Aspects of ecological anatomy of *Traganum nudatum* Del. (Amaranthaceae) from the Northeast of the Algerian Sahara

Mohamed Elhafed Kherraze[1,2*], Mohamed Belhamra[3], Marius-Nicuşor Grigore[4]

[1]Department of Ecology and Environment, Faculty of Science and the Natural and Life Science and Sciences of Earth and the Universe, University of Abou Bakr Belkaid, Tlemcen 13000, Algeria
[2]Scientific and Technical Research Center of Arid Areas (CRSTRA), Biophysical Station, 3240 Nezla, Touggourt, Algeria
[3]Department of Agronomy, Faculty of Science and the Natural and Life Sciences, University of Mohamed Kheider, Biskra, Algeria
[4]Alexandru Ioan Cuza University, Faculty of Biology, Iaşi, Romania

ABSTRACT This study focuses on the anatomical strategies developed by the *Traganum nudatum* Del., prevalent in the Algerian Sahara, particularly in the region of Oued Righ, which allows to this species to survive in a harsh environment (aridity and salinity). The anatomical structure of this species was studied using fresh materials (roots, stems and leaves). These materials have been collected from several individuals in different saline habitats. Some interesting features such as successive cambia phenomenon, calcium oxalate crystals, Kranz anatomy (salsoloid subtype), succulence, low stomata density, low stomata index, the presence of the papillae, paracytic stomata and other structures have been noticed. We can conclude that the ecological significance of evidenced adaptations by *T. nudatum* is supported in this article by the analysis of adaptations of other species belonging either to the Amaranthaceae or to other botanical families; and that this adaptation has no link with botanical families. In these species, the key adaptation is the ability to maintain growth processes and water saving under difficult living conditions (high summer temperatures or salty soils), regardless of the evolutionary level of the taxon. **Acta Biol Szeged 62(1):25-36 (2018)**

KEY WORDS
Kranz anatomy
photosynthesis C4
strategy
successive cambia
succulence

ARTICLE INFORMATION
Submitted
25 December 2017.
Accepted
30 March 2018.
***Corresponding author**
E-mail: kherrazetggt@gmail.com

Introduction

Natural rangelands in North Africa cover an area of 130 million hectares (Ben M'hamed 1990). The flora of these rangelands, important for its specific and infra-specific diversity, is typically Mediterranean with a highly developed Ibero-Maghreb element (Le Houérou 1980). Iranian-Turanian and Saharan-Sindien species are restricted to arid and desert regions. A number of genera of Amaranthaceae family are important components of the flora and vegetation of the arid to semi-arid saline environments as well as agricultural habitats in temperate and subtropical regions (Keshaavrzi and Zare 2006; Grigore 2012; Grigore et al. 2014; Safiallah et al. 2017). Amaranthaceae includes 110 to 166 genera with 1700 species (Cuenoud et al. 2002; Safiallah et al. 2017). It is one of the most interesting families in terms of having species with a great diversity the structure of carbon assimilating organs with different types of photosynthesis C_3 or C_4 (Grigore and Toma 2007; Grigore et al. 2014; Grigore and Toma 2017). The genus *Traganum* Delile, which belongs to the subfamily Salsoloideae, is represented in Amaranthaceae family by a perennial plant species, *Traganum nudatum* Delile (Fig. 1).

Traganum nudatum Del. is a Saharan-Sindien species, non-threatened, classified as "C" (IUCN 2005) and is listed on the floristic list of several protected sites listed by the UNEP World Conservation Monitoring Center (UNEP), (United Nations Environment Programme). It is a xerohalophyte species, chamaephyte (Nedjimi et al. 2012), cosmopolitan (Mroczek 2015), widespread throughout North Africa and Asia (IUCN 2005). Beside other plants belonging to the Amaranthaceae family that have a therapeutic use, such as *Cornulaca monacantha* Del., and *Haloxylon articulatum* Boiss. *Traganum nudatum* is a plant used in the traditional medicine of southern Algeria against diarrhea, wound, rheumatism, dermatitis, otitis, hemorrhoids and back pain especially low back pain. The used part is the leaf, and its mode of use is by

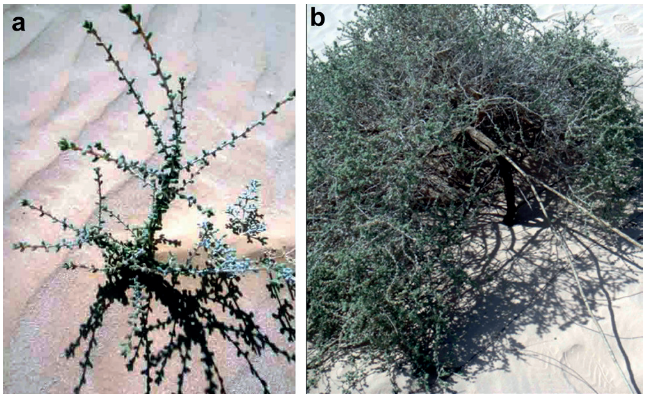

Figure 1. General view of *T. nudatum*. (a) young plant (flexible); (b) adult plant (curved and rigid).

compressed maceration, powder, or pomade (Ould El Hadj et al. 2003; Kalla 2012). Thus, the *Traganum nudatum* with other species such as *Aristida pungens*, *Zilla spinosa*, *Calligonum comosum*, *Anabasis articulata* and *Limoniastrum guyonianum* are considered to be among the most palatable species in previous studies conducted in the camel rangeland (Chehma 2006; Longo-Hammouda et al. 2007; Chehma et al. 2010; Lakhdari et al. 2015). The consumed parts are leaves and tender twigs (Bouallala et al. 2011).

The pastoral areas are dominated by steppes with variable physiognomy (grasses, chamaephyte, halophytes) (Le Floc'h 1995). Several ecological constraints of Mediterranean ecosystems can influence the physiognomy and physiology of plants. The appearance and structures that characterize certain groups of plants summarize to a large extent their ecological and physiological adaptations. *Traganum nudatum* is no exception to this rule, because of the typical structural features of the lands that distinguishes it from other groups of plants. It develops almost all important xerophytic devices to cope with the various hazards of nature such as water scarcity, heat and salt stress. These constraints are extremely detrimental to plant growth and development. Morphological and anatomical modifications of the plant organs can minimize the harmful effects of salt stress (Poljakoff-Mayber 1988; Grigore and Toma 2017).

Morphological, micromorphological and anatomical characteristics are important features, which can be implicated in taxonomical diagnosis, as well as in explaining ecological conditions (Safiallah et al. 2017). Despite the pastoral and medicinal importance of *Traganum nudatum* mentioned above, few anatomical studies have been conducted on this plant species, which led us to perform an anatomical study on the roots, stems and leaves in order to gain a global understanding of the adaptation strategy adopted by *T. nudatum*, facing the hostility of the Saharan habitats, by defining some anatomical characters all by referring in the explanation to previous studies that have been carried out on species.

Materials and Methods

Study area

The study area is located in the region of Oued Righ in the north-east of the Algerian Sahara (between 32° 54' to 39° 9' N, and 05° 50' to 05° 75' E; Fig. 2). The climate is described as Mediterranean hyperarid. The average annual rainfall does not exceed 70.3 mm (1975-2013). These insufficient rains are associated with a significant irregularity of the rains regime and a considerable inter-annual variability, which lead to a period of severe and long drought (Fig. 3). The average annual temperature is 28.47 °C. The average annual relative humidity is around

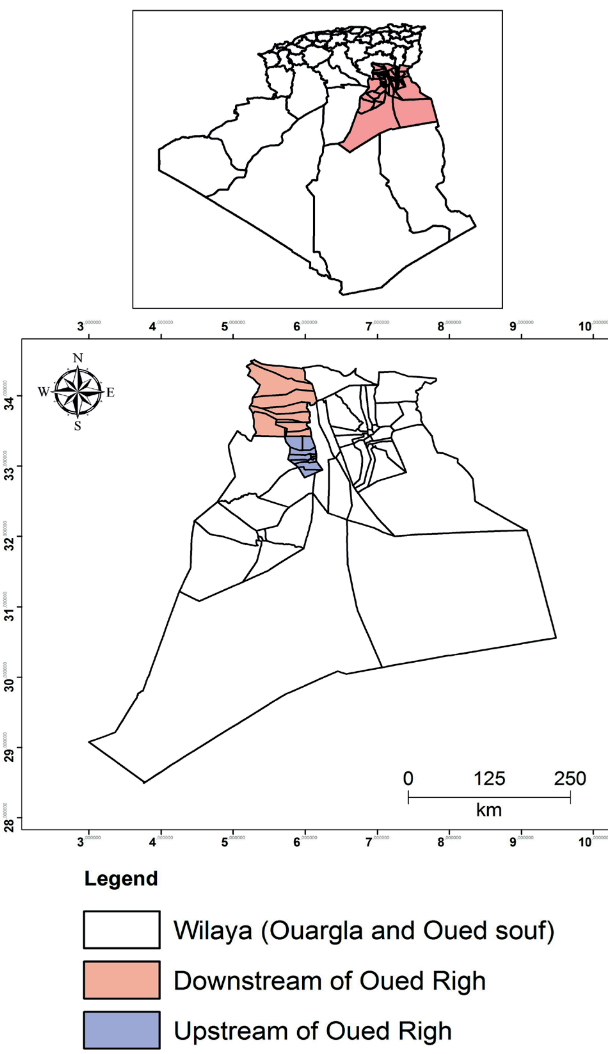

Figure 2. Map indicating the study region.

48%. The average annual evaporation is 237.96 mm, a Saharan bioclimatic stage with a mild winter. Due to the low cloudiness, sunlight in the Sahara is relatively strong and has a drying effect by increasing the temperature (Ozenda 2004).

The soils of the Saharan zone of Algeria contain significant amounts of soluble salts. Their accumulation is due to the rarity of rains that do not penetrate deeply into the soil to cause appreciable infiltration (Halilet 1998). Sogreah (1971) and Abid (1995) define the origin of soils in the Oued Righ region as alluvial, colluvial and Aeolian. The upstream portion (Touggourt) of Oued Righ is composed of shallow Aeolian sandy soils with gypsum crust and the downstream part (Djamaa) of aebral sandy soils deeper with encrustation of more recent gypsum (Mtimet and Hachicha 1998) the soils become hydromorphic all go down (El M'ghiar) in the super-salty depressions composed of fine alluvium.

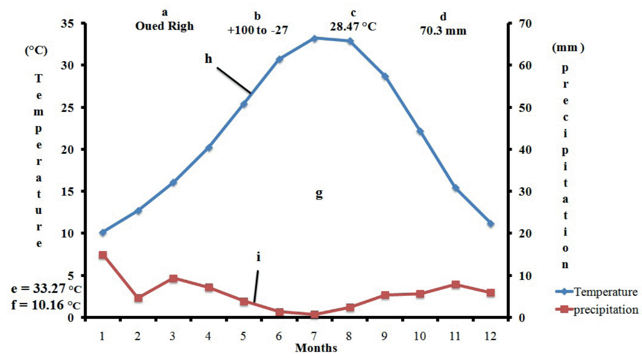

Figure 3. The climatic diagram of Oued Righ. (a) region; (b) the altitude changes gradually from + 100 m to El Goug (the upstream of Oued Righ) to - 27 m in the middle of the chott Méroune (the downstream of Oued Righ); (c) mean annual temperature (°C); (d) mean annual precipitation (mm); (e) the highest temperature average for the hottest month (°C); (f) the lowest temperature average for the coldest month (°C); (g) dry period; (i) temperature curve; (h) precipitation curve.

Sample collection and processing

In the basis of representativity, indicating the presence of several individuals of the same species at the same place (Gounot 1969), we have chosen a most dominant and abundant spontaneous species (Ozenda 1991), known by the dromedary breeders of the region under the name "Damrane", it is *T. nudatum*, belonging to the Amaranthaceae family. To carry out the various observations, cuts and analysis, we collected the plant material on about ten individuals developed in saline soil during the year 2016-2017.

The fresh material (roots, stems and leaves) was fixed and stored in alcohol (70%), according to the usual procedures. A freehand and with a sharp blade, we made very thin cross-sections on the stem in the middle part and on the roots 2 cm from the root apex, also on leaves located at the median of the twigs. These materials were treated with sodium hypochlorite for 10-15 min to empty the cells of their contents, followed by extensive washing with water, followed by rapid washing in 1% diluted acetic acid. The staining was performed in duplicate with methyl green (10 min), followed by water washing and Congo red (for 15 min), followed by washing with water (Ben Dob and Khouildat 2016). Once stained, the preparations were observed and photographed using Motic Digital light microscope (DMB1-2 MP, Motic Instruments, Xiamen, China).

Stomatal density (DS) was calculated according to Timmerman (1927) by the ratio of the number of stomata per unit area on the lower or upper faces of the leaves. By the formula: DS (Stomata per mm^2) = Number of stomata/area. Stomatal index (IS) was calculated according to the method described by Meidner and Mansfield (1968) using the value of (IS) per unit area given: IS (%) = [S/(S + E)] * 100, where, (S) is the number of stomata per unit area and (E) the number of epidermal cells for the same unit. The number of stomata and the number of epidermal cells on both sides of the leaf were counted in a 1 mm^2 area.

Results and Discussion

The observation of anatomical sections made at the root level of the species shows a successive cambia (additional) phenomenon, which is formed of incomplete concentric rings of xylem tissue, phloem tissue and cambia (Fig. 4a). This formation gives to the root the lignified aspect and it offers an ecological advantage in the conditions of water stress. Grigore and Toma (2007; 2017) found in halophytic chenopods, especially in the tertiary structure of organs that are affected by the successive cambia phenomenon, a huge amount of lignin. They have also suggested that lignin may be an element of cellular resistance against high osmotic pressure within the body of the plant (Grigore and Toma 2007; 2017). Robert et al. (2011) state that successive cambia is an important anatomical feature of wood, partially explaining the distribution of ecological species, also the appearance of species in extremely high salinity conditions such as the species of *Avicennia marina*. This phenomenon has also been recorded in stems and roots of the species *Sesuvium portulacastrum*, *Trianthema portulacastrum* and *Boerhaavia diffusa* (Patil et al. 2016).

The stele (central cylinder) of the root is wider than the cortex (Fig. 4b), it is a characteristic of the roots of the plants vegetating in salty deserts, and they seem to reduce their cortex in order to obtain a short distance between the epidermis and the central cylinder (Wahid 2003). This pattern was similar to some of the Amaranthaceae

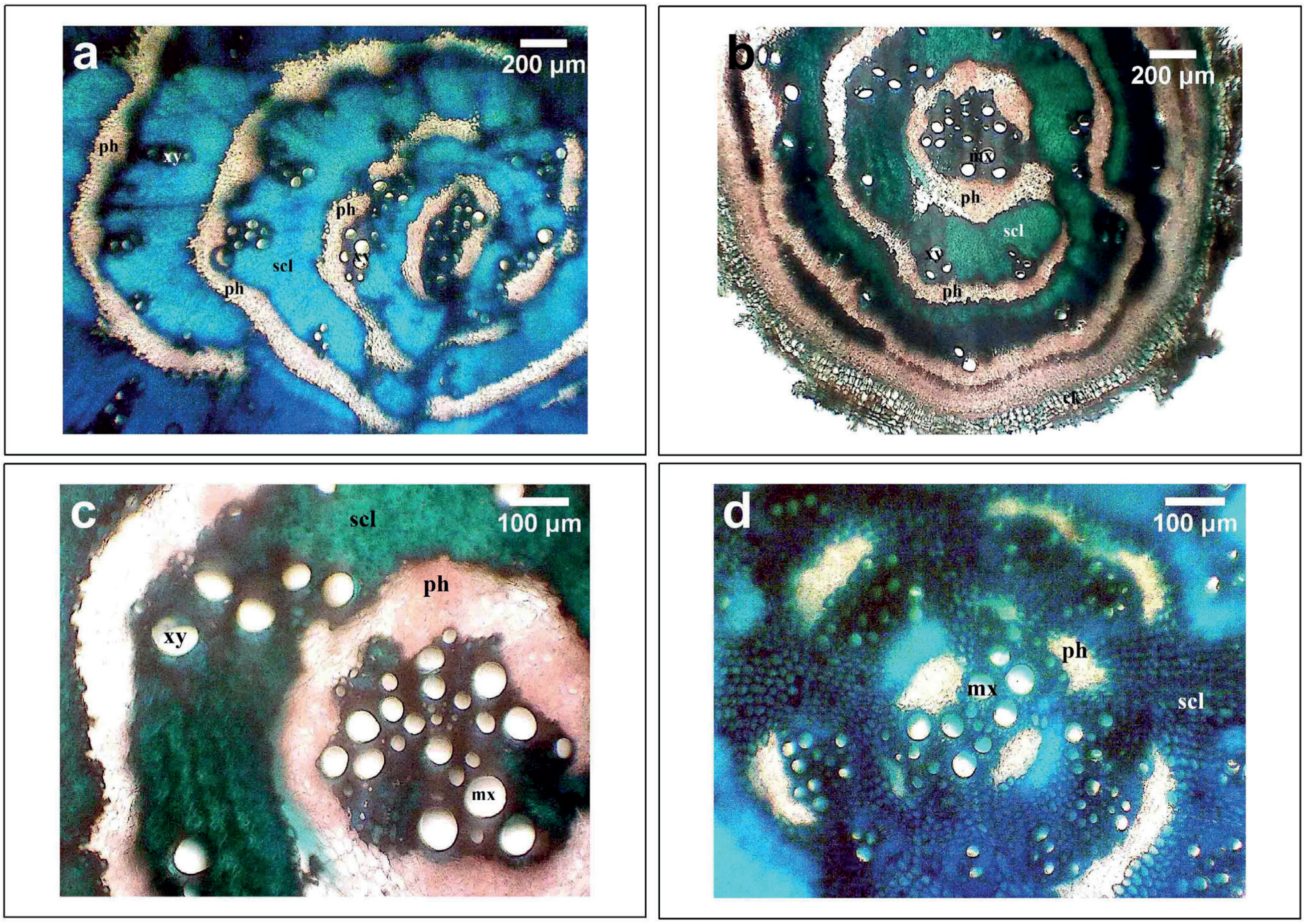

Figure 4. Cross-sections through the root of *T. nudatum*. (a) showing the successive cambia; (b) showing a wider central cylinder; (c) showing larger and more numerous metaxylem vessels; (d) showing the massive sclerification of the central cylinder (stele). xy: xylem; ph: phloem; ck: cork; mx: metaxylem; scl: sclerenchyma.

halophytes such as *Atriplex tatarica*, *Suaeda maritima* and *Camphorosma annua* (Grigore and Toma 2007). On the analyses slide (Fig. 4b), there is an interesting disposition of additional cambia products in a continuous spiral-like ring, with conjunctive tissue located between it.

The vessels of the metaxylem which are in fact the xylem vessels carrying the sap appear at the same time more widened in diameter and more numerous (Fig. 4c), which could facilitate the circulation of the water, as has been reported by Zhu et al. (2000) in *Puccinellia tenuiflora*, a highly salt tolerant species. Hameed et al. (2010) suggested that increasing the surface of metaxylem plays an important role in water conduction, and assimilates, especially in adverse salt conditions. This has been confirmed in rice (Datta and Som 1973), *Kandelia candel* (Hwang and Chen 1995), *Ziziphus lotus* (Awasthi and Pathak 1999), and *Arabidopsis thaliana* (Baloch et al. 1998). The same appearance was also observed in the genera of *Atriplex* (larger in *A. halimus*, narrower and numerous in *A. nummularia*, and wider and more numerous in *A. canescens* subjected to salt stress) (Mâalem 2011). It should also be underlined that a sclerification invades the entire mass of the central cylinder, where it can be observed that its center was previously completely occupied by metaxylem vessels (Fig. 4d). The cell walls of the sclerenchyma have thick secondary layers. This thickness was based on cellulose, hemicellulose and lignin, which will give our species hardness and rigidity (Jarvis 2012). The analyses of Chehma et al. (2010), which were carried out on the chemical composition of 21 spontaneous perennial plants from the northern Algerian Sahara, as well as the chemical analyzes of Bouallala et al. (2011) on the main plants grazed by the dromedary of the Algerian Western Sahara confirms our observation. According to Chehma et al. (2010), the dry matter percentage of crude cellulose in *T. nudatum* was 32.8% DM and 27.33% DM found by Bouallala et al. (2011) in the same species. According to Bouallala et al. (2011) the raw cellulose richness of Sa-

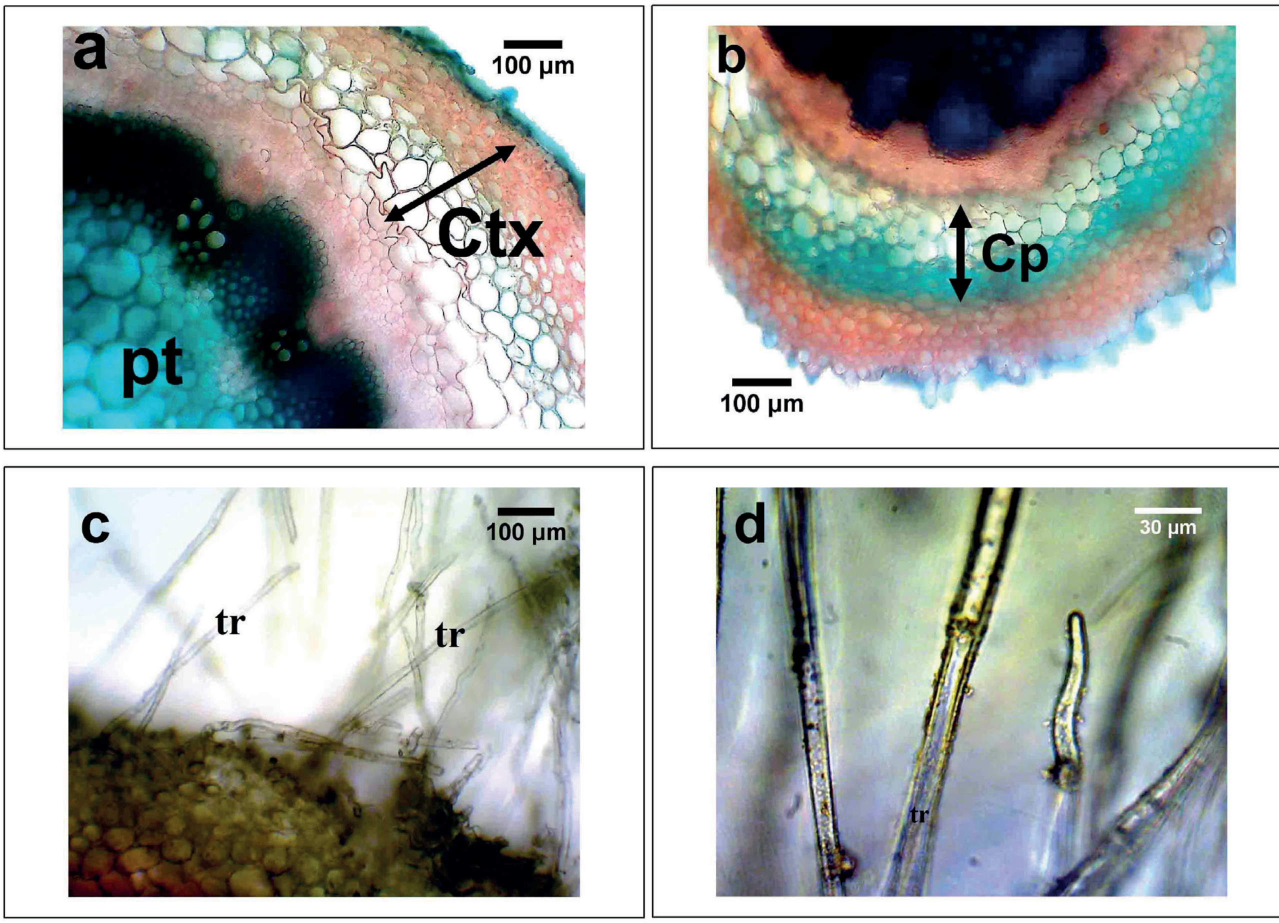

Figure 5. Cross-sections through the stem of *T. nudatum*. (a) showing the reduction of the cortex compared to the central cylinder (stele) and lignification of the pith; ctx: cortex; pt: pith; (b) showing the lignification of cortical parenchyma; cp: cortical parenchyma; (c, d) showing the multicellular trichomes, thread-like, on the epidermis of the stem; tr : trichome.

haran plants is related to their adaptation mechanism. Jarrige (1981) and Demarquilly (1982) point out that the increase in temperature stimulates the lignifications of supporting tissues. Grigore and Toma (2017) consider that all implications related to successive cambia could be related to an increased internal surface, if considering only the high capacity of retention and "storage" of the saltwater in root and stem. On the other hand, the cork outward the root could also delay water absorption. Therefore, salts penetrate slowly in roots, but once arrived there, they would be dispersed in this increased surface. Literally, the water distribution to the rest of the plant's organs seems to be "delayed." Increasing this surface would inevitably mean a dispersion area for salts, which are also diluted, thus these being ultimately less harmful to the plant. Undoubtedly, the number and diameter of xylem vessels may play a role in this mechanism.

We found well developed collenchyma tissue under the epidermis of the stem; it may confer to the stem good elasticity, great resistance to flexion, traction, and good support, since the cells of the collenchyma have very thick cellulosic walls (Zaffran 1998). This anatomical observation corresponds perfectly to the state of aspect of the studied species. The plant is more erect and more flexible in the case of young plants and becomes curved and lignified in adulthood, which confers a form ranging from large tufts to that of a shrub. The elasticity may be advantageous for young plants facing intense wind usually existing in the spring period. The cortex of the stem appears to be reduced in thickness, this corresponding to an adaptive value (Fig. 5a). In fact, this adaptation has been observed in the twigs of *Salsola vermiculata*, *Rhanterium adpressum* (Houari et al. 2012) and even in the alfalfa stem (Zaffran 1998). However, in some halophytic species such as *Euphorbia guyoniana* and *Petrosimonia opposittifolia*, the cortex is very broad with a cortical parenchyma consisting of large water storage cells which gives to these species a succulent appearance (Houari et al. 2012; Grigore

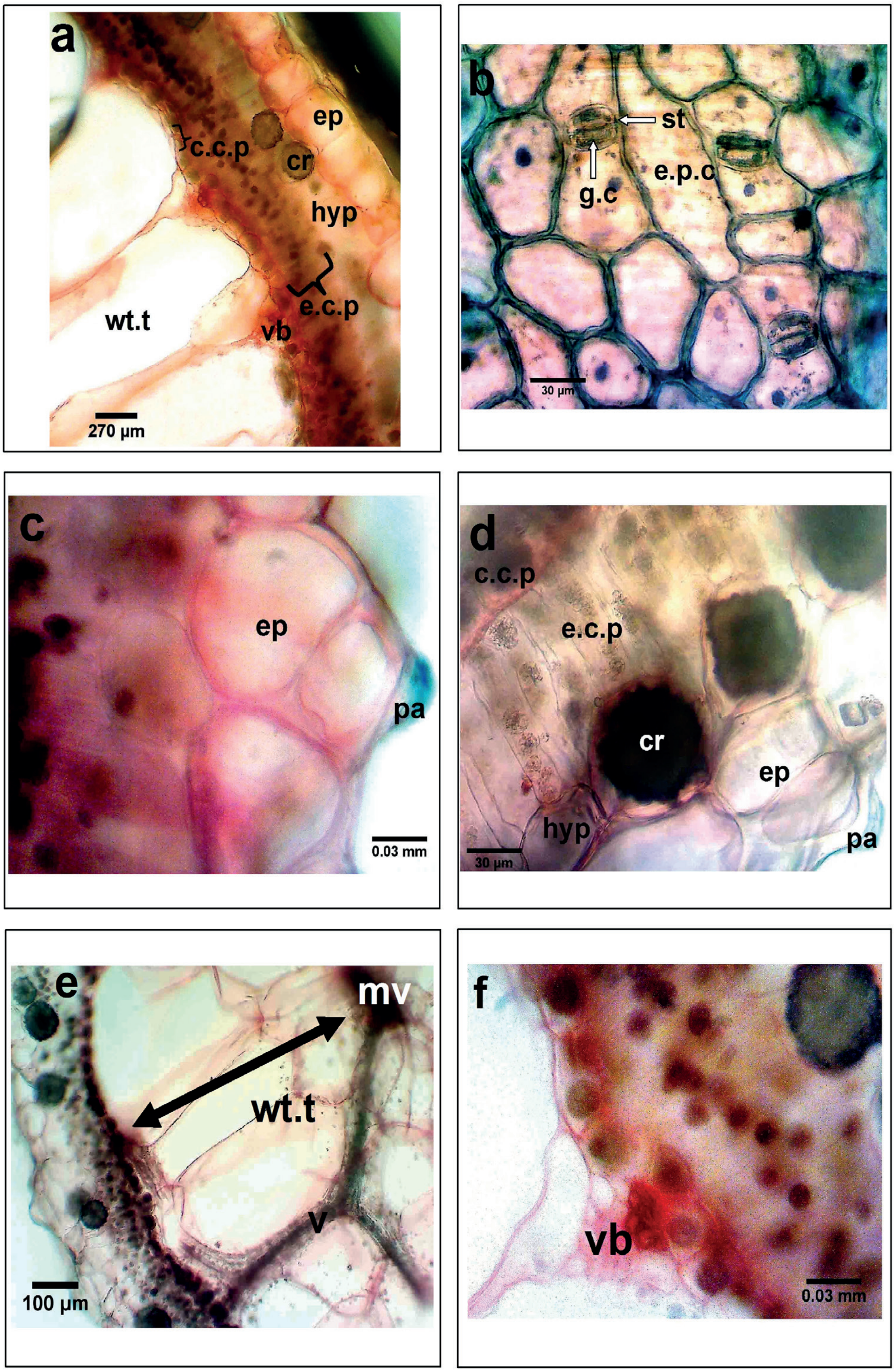

Figure 6. Cross-sections of C4 leaf in *T. nudatum*. Fig. 6a. general view (arrangement of cellular tissues in the lamina); Fig. 6b. shows the arrangement of stomata (paracytic type); Fig. 6c. papilla non aiguë in the epidermis; Fig. 6d. shows the organization of the chlorenchymatic (assimilative) parenchyma in two layers (external (e.c.p) and internal (c.c.p) and the presence of a hypodermis rich in crystals of calcium oxalate (twin form); Fig. 6e. shows large cells of water storage tissue occupying the central area of the leaf and the arrangement of the ribs; Fig. 6f. shows the contact of a vascular bundle with the inner layer (c.c.p) (structural model - known as Kranz anatomy of salsoloid type). ep: epidermis; hyp: hypodermis; e.c.p: elongated cell parenchyma; c.c.p: cubic cell parenchyma; cr: calcium oxalate crystals; vb: vascular bundle; wt.t: water storage tissue; st: stoma; g.c: guard cells; ep.c: epidermal cells; pa: papilla; mv: main vein; v: vein.

and Toma 2007). Another adaptive trait that appears to strengthen the organ in question (stem) is the lignification of cortical parenchyma. This lignification makes the tissues rigid and therefore provides mechanical support to the stem (Fig. 5b). According to Wang et al. (1997), under saline stress, the lignification of the apex of the stems becomes more and more pronounced in *A. prostrata*. The lignification of the pith also appears to give mechanical support to the stem (Fig. 5a). This same aspect was also observed in the pith of the American species *Atriplex canescens* subjected to salt stress (Mâalem 2011) and in the species *Smilax aspera* (Zaffran 1998).

We also evidenced the presence of multicellular trichomes, thread-like, on the surface of the epidermis of the stem of young plants (*T. nudatum*) (Figs. 5c, d). However, the stems of adult plants are completely hairless. These trichomes could have a protective function against environmental changes (*e.g.,* excessive heat). Thus, the presence of *T. nudatum* in open habitats, hot and dry in the study area may be one reason for the presence of these trichomes in young plants (*T. nudatum*) to protect the most sensitive tissues. Some authors such as Grigorev (1955) cited by Weryszko-Chmielewska and Chernetskyy (2005), Agren and Schemske (1993), Nabors (2008) reported that these trichomes protects plants against drought (reducing the intensity of transpiration), prevent the increase in temperature, maintain moisture at surface level (stem or leaf), also they have a defensive function (insect attack or herbivores), it may be a reason more to the presence of these trichomes in young seedlings. In addition to these previous observations on the stem and the root, the anatomical characteristics of the *T. nudatum* leaf are expressed by several criteria related to the mode of adaptation to the Saharan environment (Fig. 6a). A structure evidenced at the level of the epidermis, presence of a paracytic-type stomata arrangement, where two lateral secondary cells oriented parallel to the guard cells (Fig. 6b), as described by Carpenter (2005) based on the work of Dilcher (1974). This paracytic type reflects the xerophytic features of the species (Smail-Saadoun 2005a; Kadi-Bennane et al. 2005). Kadi-Bennane et al. (2005) have reported that the increase in paracytic type frequency is influenced by climatic conditions, this fact is demonstrated with the species of the genus *Pistacia*, where the authors found a positive correlation between the increase of the frequency of the paracytic type and the increase of the degree of aridity of the station. The higher the degree of aridity of the station, the more the frequency of the paracytic type increases (the Emberger coefficient of the stations: Ain Oussera, Messaad and Taissa respectively 23, 16 and 10). We compare these coefficients with those of the region of Oued Righ which is 6.63; we see that these results of these authors support our observation. This type of stomata arrangement (paracytic) has also been found in spontaneous species of the northern Sahara such as *Limoniastrum guyonianum* (Plumbaginaceae), *Anabsis articulata* (Amaranthaceae) and *Pituranthos chloranthus* (Apiaceae) (Benghersallah 2013). Other anatomical features evidenced in the leaf can be correlated with the peculiarities of the environment (very long dry period and very high summer temperature) where the plant grows, which would explain the required presence of a low stomatal density (01-03 stomata/mm²) with a low stomatal index (by means of 11.6%). This observation was supported by the work of Finsinger et al. (2013), who suggested that variation in stomatal frequency (stomatal density and stomatal index) was related to regional environmental conditions (climatic). Kadi-Bennane et al. (2005) have reported that stomatal density is influenced by climatic conditions, this fact is demonstrated with species of the genus *Pistacia* where the authors found a correlation between stomatal density and aridity degree of the station. Other authors such as Flowers et al. (1986), Bray and Reid (2002) reported decreasing the number of stomata and stomatal index at increased salinity of the soil. Indeed, the plant by using this strategy of reducing the number of stomata decreases the loss of water by evaporation (Gorenflot 1980).

In addition, we were able to evidence at the level of the leaf, the presence of an eminence in the form of a papilla, non-acute of a length varying between 0.02 mm and 0.50 mm at the level of the epidermis (Fig. 6c). A subepidermal layer, hypodermis, rich in calcium oxalate crystals in the form of twins (Fig. 6d) has been evidenced. There are two types of chlorenchyma located under the epidermis and hypodermis: an external chlorenchyma consisting of elongated cells of cylindrical form, and an internal chlorenchyma made of cubic cells (Fig. 6d). A developed water storage tissue occupies the central zone (Figs. 6e, 7a), and it is formed of large cells with thin wall, in which the nervures radiate in all directions, which observed to anastomose in a network under the inner layer of cubic cells (Figs. 6f, 7b).

The observation of the papillae was also recorded at a cross section of the stem in a hygro-halophytic species collected from different salt marshes of Iran: the *Halostachys belangeriana* (Moq.). Botch with a length varies between 0.04 mm and 0.06 mm and the stem of *Halocnemum strobilaceum* (Pall.) M. Bieb with a length of about 0.01 mm (Keshaavrzi and Zare 2006). Thus, our observations to other tissues were consistent with the observations of Smail-Saadoun (2005b) at the level of *T. nudatum* leaf harvested in the Béni-Abbès region (Algeria).We note that the presence of the hypodermis was also able to characterize 8 species of *Chenopodiaceae* on the 14 species studied by Saadoun (2005b), and the assimilating parenchyma structure also made it possible

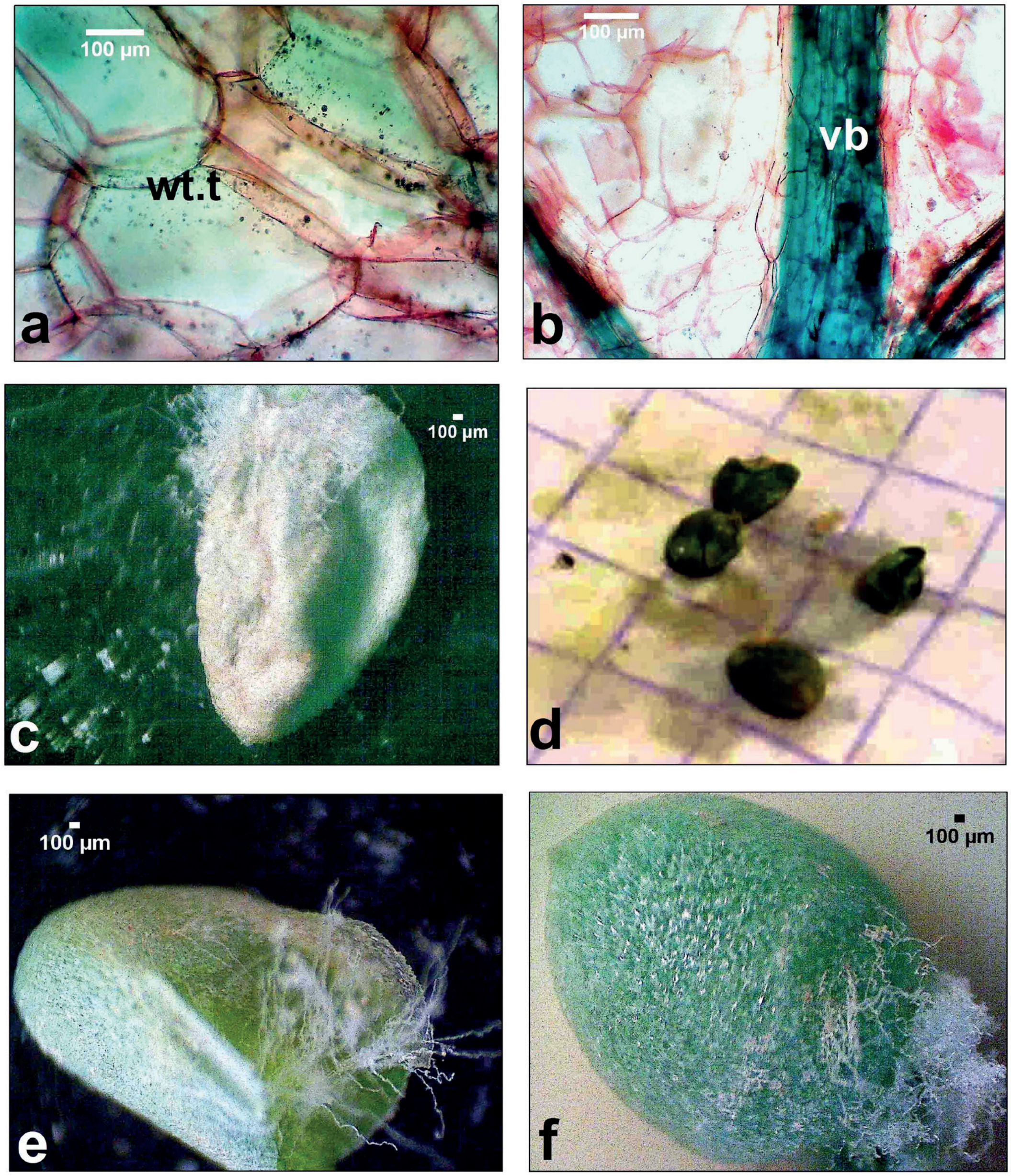

Figure 7. An overview of some morphological and anatomical of the leaf of *T. nudatum*. (a) a cross section shows the water storage tissue; (b) a longitudinal section shows the vascular network; (c) showing a dried leaf; (d) a millimeter paper wet with water sap from compressed leaves; (e, f) showing the succulent appearance of leaves.

to characterize 11 species of Chenopodiaceae studied by the same author. These interesting structures that have been evidenced at the leaf level can be correlated with the peculiarities of the environment, in which the *T. nudatum* grows. Indeed, in the case of excessive heat, the papillae structure of the epidermis of the *T. nudatum* leaf contributes to disseminate the heat. According to Collin (2001), the high heat can have a negative impact on the metabolism of the plant. The same author also argues that the presence of papilla in some cacti (e.g., *Mammilaria* and *Echinopsis*) helps dissipate heat.

The presence of the hypodermis can reinforce the insulating and mechanical characteristics of the epidermis. A particular function of this tissue has been suggested by Pautov and Telepova-Texier (1999) that the hypodermis ensures the absorption of most of the radiation and thus reduces the heat load on the plant, as it participates in the transport of substances in the subepidermal layer of the mesophyll and also, it protects the foliar blanks against dehydration. Another function of the hypodermis reported by Smail-Saadoun (2005b) is that the hypodermis separates the assimilative parenchyma from the surface of the leaves. The richness of the hypodermis by calcium oxalate crystals could be correlated with plant metabolism. It is an end product of metabolism (Franceschi and Horner 1980) and can also be considered as an effort to maintain ionic balance (Franceschi and Horner 1980; Grigore et al. 2014). Some authors such as Paupardin (1965) and Calmes and Piquemal (1977) cited by Vintéjoux and Shoar-Ghafari (1985) working on other plants, respectively, vine and hawthorn grown *in vitro*, proved that calcium oxalate could be reused in the tissue metabolism of these plants. These last results will encourage us to realize in the future studies in this direction on *T. nudatum*. The organization of assimilating parenchyma in *T. nudatum* in two adjoining and different layers: outer layer with elongated cells (e.c.p) (also called external chlorenchyma) and inner layer with cubic cells (c.c.p) (also called internal chlorenchyma) refer to a specific functional anatomy. This examination is confirmed by the work of Smail-Saadoun (2005b), Muhaidat et al. (2007), Houari et al. (2012), Grigore and Toma (2007), on several genera: *Kochia, Camphorosma, Petrosimonia, Atriplex, Anabasis, Salsola* and *Bassia*.

According to Ting (1975) and Depuit (1978), the species bearing this organization correspond to the photosynthetic pathway of the C_4 type (Figs. 6a, 6d and 6f). According to the work of Smail-Saadoun (2005b), Muhaidat et al. (2007), Houari et al. (2012), the cubic cells of the inner layer are characterized by a greater number of chloroplasts and mitochondria than the elongated cells of the outer layer. In addition, many vascular elements have been observed that anastomose in a network under the cubic layer (presence of vein contact with the inner layer), which seems to be a more specialized layer than the outer layer of elongated cells. According to Muhaidat et al. (2007), this structural model – known as Kranz anatomy – belongs to salsoloid subtype. It has been shown at the level of the species *Salsola kamarovii* (Amaranthaceae) by the same author. This internal architecture physically divides the biochemical events of the C_4 pathway into two stages. (I) Beforehand atmospheric CO_2 is incorporated into four-carbon acids, hence the name C_4 photosynthesis. This incorporation will take place at the level of the external layer (e.c.p), (II) decarboxylation of this C_4 acid and the released CO_2 is re-fixed by Rubisco specific to the internal tissue (c.c.p) (Muhaidat et al. (2007). This bi-phasic system C_4 seems to be a good adaptation to drought since the Calvin cycle and carbohydrate synthesis occur at the level of the internal cells (c.c.p) of the parenchyma assimilator away from the heat and in the vicinity of the vessels, which implies an easier water supply and fast evacuation of carbohydrates (Heller et al. 1993; Sage 2004; Smail-Saadoun 2005b; Houari et al. 2012). In addition to the importance of the successive cambia phenomenon at the root level, or to Kranz anatomy from leaf level, there is also another more important phenomenon at the level of the *T. nudatum* leaf. It is the phenomenon of succulence, which is correlated with local environmental factors. Thus, *T. nudatum* was collected from saline soil, which would explain the necessary presence of water storage tissue at the leaf and its globular form (Fig. 7). This parenchyma tissue appears very thick compared to other tissues of the leaf. This thickening of this tissue could be due to the salinity of the soil. Several published data show that salinity increases succulence in plants such as *Atriplex patula* of the Amaranthaceae family (Longstreth and Nobel 1979), *Cakile maritima* of the Brassicaceae family (Debez et al. 2006), *Nitraria retusa* of the Nitrariaceae family (Boughalleb et al. 2009) and even in glycophytes such as *Gossypium hirsutum* of the Malvaceae family (Longstreth and Nobel 1979) and *Hordeum vulgare* of the Poaceae family (Kiliç et al. 2007), are similar to our observation. This succulence phenomenon makes it possible to slow the rise in temperature of the exposed leaves, because it is more difficult to heat a volume of spherical water than the same volume of water spread as is the case in a non-succulent leaf (Collin 2001). In addition, succulence exerts a dilution effect on salts accumulated in *T. nudatum* leaves, which allows the leaves to cope with higher amounts of salt. Debez et al. (2006) showed that succulence is one of the adaptations to increased salinity. According to Larcher (1986) cited by Mantovani (1999), a plant would be more resistant to drought if it possessed a large capacity of water storage, besides low percentages of transpiration.

Conclusion

The present anatomical study of *T. nudatum*, conducted for the first time in the north-east of the Algerian Sahara, revealed a certain adaptation of the species to a hostile environment (severe aridity and saline soil); thus, *T. nudatum* has shown two very important strategies: tolerance and avoidance. In the case of tolerance, the succulence of the lamina is considered, to be one of the main factors involved in salt tolerance and secondly the presence of calcium oxalate at the level of the hypodermis of the leaf can play a metabolic role conferring salt resistance. In the case of the avoidance strategy, there is a reduction of transpiration, which is an essential element of drought resistance since it allows the maintenance of a high water potential. It is also translated by the reduction of the thermal load of the leaf surface. The reduction of transpiration and the thermal load are obtained by reduction and protection of the transpiring surface: reduction (density and low stomatal index, assimilative parenchyma distant from the leaf surface, presence of trichomes in the epidermis of the stem) and protection (presence of the papilla and hypodermis and of the globular form due to succulence), in addition, by the specific arrangement of stomata (paracytic type). Other adaptive histological characters appeared at the root and the stem for the reduction of the water and nutrient requirement by the decrease of the density of the living tissues following the presence of successive cambia phenomena and the lignification which also may act against dehydration In addition, the improvement in water uptake is achieved by increasing the number and the diameter of the metaxylem vessels, thus filling the water deficit imposed by the high salinity of the soil solution. Kranz anatomy of salsoloid subtype in *T. nudatum* is necessary for C_4 photosynthesis and contributes to an increased adaptation to drought and salinity.

References

Abid F (1995) Caractérisation des sels des sols de l'Oued Righ. Engineer dissertation, University of Batna, Algeria.

Awasthi OP, Pathak RK (1999) Effect of salinity levels on survival and anatomy of four scion cultivars budded on Indian jujube. Hort J 12(2):53-59.

Agren J, Schemske DW (1993) The cost of defense against herbivores: an experimental study of trichome production in *Brassica rapa*. Am Nat 141(2):338-350.

Ben M'hamed M (1990) Forage shrubs in North Africa-Studies of the green Belt of North Africa. ALESCO, Tunis, Tunisie.

Ben Dob H, Khouildat A (2016) Action de la salinité et de l'acide salicylique sur le comportement physiologique et anatomique des plantules d'*Atriplex halimus* L. et *Atriplex canescens* (Pursh) Nutt. PhD Thesis. Université Kasdi Merbah, Ouargla.

Bouallala M, Chehma A, Bensetti M (2011) Variation de la composition chimique de principales plantes broutées par le dromadaire du Sud-Ouest Algérien. LRRD 23(5):1-9.

Baloch AH, Gates PJ, Baloch GM (1998) Anatomical changes brought about by salinity in stem, leaf and root of *Arabidopsis thaliana* (L.) Heynh (thale cress). Sarhad J Agric Pak 14:131-142.

Benghersallah N, Elhadi K (2013) Réponse anatomique à la sécheresse de quelques plantes spontanées du Sahara septentrional. Master Académique. Université Kasdi Merbah, Ouargla.

Bray S, Reid DM (2002) The effect of salinity and CO2 enrichment on the growth and anatomy of the second trifoliate leaf of *Phaseolus vulgaris*. Can J Bot 80(4):349-359.

Boughalleb F, Denden M, Tiba BB (2009) Anatomical changes induced by increasing NaCl salinity in three fodder shrubs, *Nitraria retusa*, *Atriplex halimus* and *Medicago arborea*. Acta Physiol Plant 31(5):947-960.

Cuénoud P, Savolainen V, Chatrou LW, Powell M, Grayer RJ, Chase MW (2002) Molecular phylogenetics of Caryophyllales based on nuclear 18S rDNA and plastid *rbcL*, *atpB*, and *matK* DNA sequences. Am J Bot 89(1):132-144.

Chehma A (2006) Catalogue des plantes spontanées du Sahara septentrional algérien. Ed Dar El Houda, Algérie 140p.

Chehma A, Faye B, Bastianelli D (2010) Valeurs nutritionnelles de plantes vivaces des parcours sahariens algériens pour dromadaires. Fourrages 204:253-256.

Carpenter KJ (2005) Stomatal architecture and evolution in basal angiosperms. Am J Bot 92(10):1595-1615.

Collin P (2001) L'adaptation au milieu chez les plantes vasculaires. L'Année Biol 40:21-42.

Calmés J, Piquemal M (1977) Variation saisonnière des cristaux d'oxalate de calcium des tissus de Vigne vierge. Can J Bot 55(15):2075-2078.

Datta SK, Som J (1973) Effect of salinity on structural-changes in stem of rice varieties. Indian J Agric Sci 43(6):614-617.

Demarquilly C (1982) Influence des facteurs climatiques sur la composition et la valeur nutritive de l'herbe. In: Actions du climat sur l'animal au pâturage, Séminaire de Theix. Éd., INRA publications, Versailles 49-63.

Dilcher DL (1974) Approaches to the identification of angiosperm leaf remains. Bot Rev 40(1):1-157.

Depuit DJ (1978) Photosynthesis and respiration of plant. In the arid ecosystem. Cambridge: Cambridge University Press, 509-36.

Debez A, Saadaoui D, Ramani B, Ouerghi Z, Koyro HW, Huchzermeyer B, Abdelly C (2006) Leaf H^+-ATPase activity and photosynthetic capacity of Cakile maritima under increasing salinity. Environ Exp Bot 57(3):285-295.

Finsinger W, Dos Santos T, McKey D (2013) Estimating variation in stomatal frequency at intra-individual, intra-site, and inter-taxonomic levels in populations of the *Leonardoxa africana* (Fabaceae) complex over environmental gradients in Cameroon. CR Geoscience 345(7-8):350-359.

Flowers TJ, Hajibagheri MA, Clipson NJW (1986) Halophytes. Q Rev Biol 61(3):313-337.

Franceschi VR, Horner HT (1980) Calcium oxalate crystals in plants. Bot Rev 46(4):361-427.

Grigore MN (2012) Romanian Salt Tolerant Plants. Taxonomy and Ecology Edit. Tehnopress, Iasi.

Grigore MN, Ivanescu L, Toma C (2014) Halophytes. An integrative anatomical study. Springer, Cham, Heidelberg, New York, Dordrecht, London.

Grigore MN, Toma C (2007) Histo-anatomical strategies of Chenopodiaceae halophytes: adaptive, ecological and

evolutionary implications. WSEAS Trans Biol Biomed 4(12):204-218.
Grigore MN, Toma C (2017) Anatomical Adaptations of Halophytes. A Review of Classic Literature and Recent Findings, Springer.
Grigorev JS (1955) Comparative ecological investigations of higher plant xerophilization. AN SSSR, Moscov-Leningrad. (In Russian).
Gounot M (1969) Méthodes d'étude quantitative de la végétation. Masson, Paris.
Gorenflot R (1980) Biologie végétale. Plantes supérieures: appareil végétatif. Masson, Paris.
Halillet MT (1998) Étude expérimentale de sable additionnée d'argile Comportement physique et organisation en condition saline et sodique. PhD Thesis. I.N.A.P.G, Paris.
Hameed M, Ashraf M, Naz N, Al-Qurainy F (2010) Anatomical adaptations of *Cynodon dactylon* (L.) Pers., from the salt range Pakistan, to salinity stress. I. Root and stem anatomy. Pak J Bot 42(1):279-289.
Houari KD, Chehma A, Zerria A (2012) Étude de quelques paramètres d'adaptation anatomique des principales plantes vivaces spontanées dans la région d'Ouargla (Algérie). Sécheresse 23(4):284-288.
Hwang YH, Chen SC (1995) Anatomical responses in *Kandelia candel* (L.) Druce seedlings growing in the presence of different concentrations of NaCl. Bot Bull Acad Sin 36:181-188.
Heller R, Esnault RLC (1993) Physiologie végétale. Vol. I. Nutrition, 5ème éd. Masson. Paris.
IUCN (World Conservation Union) (2005) Guidelines for using the IUCN Red List Categories and Criteria. Prepared by the Standards and Petitions Subcommittee of the IUCN SSC Red list Programme Committee. IUCN, Gland, Switzerland, and Cambridge, United Kingdom. Available: http://www.iucn.org/themes/ssc/red-lists.htm. Accessed September 2006.
Jarvis MC (2012) Sclerenchyma. eLS. Glasbow University, Glasgow, Scotland, UK DOI:10.1002/9780470015902.a0002082.pub2.
Jarrige R (1981) Constituants glucidiques des fourrages: variations, digestibilité et dosage. In Prévision de la valeur nutritive des aliments des ruminants/ouvrage collectif coordonne par C. Demarquilly, éd., INRA publications, Versailles, 13-40.
Keshavarzi M, Zare G (2006) Anatomical study of *Salicornieae dumort* (Chenopodiaceae Vent.) native to Iran. Int J Bot 2(3):278-285.
Kalla A (2012) Étude et valorisation des principes actifs de quelques plantes du sud algérien. Thèse PhD. Université Mentouri-Constantine Algérie.
Kadi-Bennane S, Ait-Said S, Smail-Saadoun N (2005) Adaptation study of three *Pistacia atlantica* Desf. ssp. *atlantica* populations (Ain Oussera - Messaad - Taissa) through stomatal complex. In: Oliveira M.M. (ed.), Cordeiro V. (Ed.). XIII GREMPA Meeting on Almonds and Pistachios. Zaragoza: CIHEAM, Opt Méd 63:365-368.
Kılıç S, Çavuşoğlu K, Kabar K (2007) Effects of 24-epibrassinolide on salinity stress induced inhibition of seed germination, seedling growth and leaf anatomy of barley. SDU Fen-Edb Fak Fen Dergisi (E-Dergi) 2(1):41-52
Le Houérou HN (1980) Fourrages ligneux en Afrique du nord. In Les fourrages ligneux en Afrique. État actuel des connaissances. Colloque sur les fourrages ligneux en Afrique, Addis Abeba, Éthiopie, 57-84.
Longo-Hammouda FH, Siboukheur OE, Chehma A (2007) Aspects nutritionnels des pâturages les plus appréciés par *Camelus dromedarius* en Algérie. Agricultures 16(6):477-483.
Lakhdari K, Belhamra M, Mayouf R (2015) Forage species preferred by dromedaries and their chemical composition in arid rangelands of Algeria. LRRD 27(10):1-10.
Le Floc'h E (1995) Les écosystèmes des zones arides du Nord de l'Afrique: orientation pour l'établissement d'un réseau de réserves de biosphère. In: Nabli MA, ed., Ouvrage collectif sur le milieu physique et la végétation. Essai de synthèse sur la végétation et la phytoécologie tunisienne- 5 et 6. Faculté des sciences de Tunis/ Agence de coopération culturelle et technique/ MAB, Tunis, 309-321.
Longstreth DJ, Nobel PS (1979) Salinity effects on leaf anatomy: consequences for photosynthesis. Plant Physiol 63(4):700-703.
Larcher W (1986) Ecofisiologia Vegetal. Curitiba: Editora Pedagógica e Universitária Ltda., São Paulo.
Mroczek A (2015) Phytochemistry and bioactivity of triterpene saponins from Amaranthaceae family. Phytochem Rev 14(4):577-605.
Mtimet A, Hachicha M (1998) Gestion durable de l'eau et du sol dans les oasis tunisiennes. In: Proceedings of the 16th ISSS World Congress. August 1998, Montpellier, France.
Meidner H, Mansfield TA (1968) Physiology of stomata. McGraw-Hill, London.
Mâalem S (2011) Étude de l'impact des interactions entre le phosphore et le chlorure de sodium sur trois espèces végétales halophytes du genre Atriplex (*A. halimus*, *A. canescens* et *A. nummularia*). PhD Thesis, Université Badji Mokhtar-Annaba, Algérie.
Muhaidat R, Sage RF, Dengler NG (2007) Diversity of Kranz anatomy and biochemistry in C_4 eudicots. Am J Bot 94(3):362-381.
Mantovani A (1999) A method to improve leaf succulence quantification. Braz Arch Biol Technol 42(1):9-14.
Nedjimi B, Beladel B, Guit B (2012) Biodiversity of halophytic vegetation in Chott Zehrez Lake of Djelfa (Algeria). Am J Plant Sci 3(11):1527-1534
Nabors MW (2008) Biologie végétale: structures, fonc-

tionnement, écologie et biotechnologies. Pearson Éducation, France, Paris.
Ould El Hadj MD, Hadj-Mahammed M, Zabeirou H (2003) Place des plantes spontanées dans la médicine traditionnelle de la région d'Ouargla (Sahara septentrional Est). Courrier du Savoir (3):47-51.
Ozenda P (2004) Flore et végétation du Sahara, 3ème éd. CNRS Paris.
Ozenda P (1991) Flore de Sahara, 3ème éd. mise à jour et augmentée. CNRS Paris.
Poljakoff-Mayber A (1988) Ecological-physiological studies on the responses of higher plants to salinity and drought. Sci Rev Arid Zone Res 6:163-183.
Patil VS, Rajput KS, Malpathak NP (2016) Comparative study on morpho-anatomy of leaf, stem and root of *Boerhaavia diffusa* L. (Nyctaginaceae) and its adulterant plants. Braz J Pharm Sci 52(3):433-442.
Pautov A, Telepova-Texier M (1999) Structure et développement de l'hypoderme dans les feuilles de Peupliers (Salicaceae). Acta Bot Gallica 146(2):123-137.
Paupardin C (1965) Sur la morphologie des tissus d'Aubépine (*Crataegus monogyllil* Jacq.) cultivés in vitro et la possibilité pour ces tissus d'utiliser l'oxalate de calcium comme substance de réserve. 90ème Congr Nat Soc Sav (Nice). C.R. II. GauthierVillars, Paris 379-389.
Robert EMR, Schmitz N, Boeren I, Driessens T, Herremans K, De Mey J, Van de Casteele E, Beeckman H, Koedam N (2011) Successive cambia: a developmental oddity or an adaptive structure? PLoS One 6(1)e16558.
Safiallah S, Hamdi SMM, Grigore MN, Sara J (2017) Micromorphology and leaf ecological anatomy of *Bassia halophyte* species (Amaranthaceae) from Iran. Acta Biol Szeged 61(1):85-93.
Sogreah S (1971) Participation à la mise en valeur de l'Oued Righ. Étude agro pédologique, Doc Poly MTPC Alger 7-36.
Smail-Saadoun N (2005a) Stomata types of *Pistacia* genus: *Pistacia atlantica* Desf. ssp. *Atlantica* and *Pistacia lentiscus* L. In: Oliveira M.M. (ed.), Cordeiro V. (ed.). XIII GREMPA Meeting on Almonds and Pistachios . Zaragoza : CIHEAM, (Options Méditerranéennes : Série A. Séminaires Méditerranéens). 13. Meeting of the Mediterranean Research Group for Almond and Pistachio, 2003/06/01-05, Mirandela (Portugal), (63):369-371.
Smail-Saadoun N (2005b) Réponse adaptative de l'anatomie des Chénopodiacées du Sahara algérien à des conditions de vie d'aridité extrême. Sécheresse 16(2):121-124.
Sage RF (2004) The evolution of C_4 photosynthesis. New Phytol 161(2):341-370.
Timmerman HA (1927) Stomatal numbers: their value for distinguishing species. Pharm J 118:241-243.
Ting IP (1975) Physiological adaptation to water stress in desert plants. In: Vernberg FJ, ed. Physical Adaptation to the Environment. Intext Educational Publishers, New York, 99-109.
Vintéjoux C, Shoar-Ghafari A (1985) Répartition et ultrastructure comparées des cellules oxalifères en vie latente et en vie active de *Spirodela polyrrhiza* L. (Lemnacées). Bulletin de la Société Botanique de France. Lettres Botaniques 132(1):25-39.
Wahid A (2003) Physiological significance of morpho-anatomical features of halophytes with particular reference to cholistan flora. Int J Agri Biol 5(2):207-212.
Wang L, Showalter A, Ungar I (1997) Effect of salinity on growth, ion content, and cell wall chemistry in *Atriplex prostrata* (Chenopodiaceae). Am J Bot 84(9):1247-1255.
Weryszko-Chmielewska E, Chernetskyy M (2005) Structure of trichomes from the surface of leaves of some species of *Kalanchoë* Adans. Acta Biol Cracov Ser Bot 47(2):15-22.
Zhu Y, Zhang Y, Hu Z, Yan S (2000) Studies on microscopic structure of *Puccinellia tenuiflora* stem under salinity stress. Grassland China (5):6-9.
Zaffran J (1998) Initiation à la biologie végétale. éd. Ellipses, Paris.