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TAXONOMIC SIGNIFICANCE OF STEM, LAMINA AND EPIDERMAL MICRO-CHARACTERS IN UNDERSTANDING CHENOPODIACEAE AND AMARANTHACEAE ALLIANCE

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Abstract

Despite the importance of Chenopodiaceae and Amaranthaceae, the two families were not thoroughly explored and discussed from taxonomic point of view, because of the limited taxonomic characters and the succulent nature of several species. The stem and lamina anatomical characteristics as well as epidermal micro-characters of 35 wild and cultivated taxa (29 of Chenopodiaceae and six of Amaranthaceae) were investigated using LM and SEM. The specific objective is to estimate the taxonomic weight of these characters in understanding the Chen-Am alliance. The obtained stem, lamina and epidermal micro-characters were considered diagnostic at generic and specific level. Two major aspects of growth: normal secondary growth (five taxa belonging to Chenopodiaceae) and abnormal growth (30 taxa of Chenopodiaceae & Amaranthaceae). Five sub-types of anomalous growth were recognized *viz*, successive cambia, medullary bundles, included phloem, included phloem with bipolar vascular bundles or included phloem with medullary bundles. Six types of lamina anatomy were recognized based on mesophyll type, size and distribution of kranz cells *viz*, atriplcoid, kochioid, kranz-ventrodorsal, salsoloid, flat-leaved salsoloid and non-kranz type. Brachyparacytic and anomocytic stomata, rhomboid crystals, platelets, fissured crust, and granules epicuticular wax were recorded. Most of the obtained data reinforce the alliance between the studied taxa of Chenopodiaceae and Amaranthaceae. The most common characters that support this alliance are anomalous secondary growth (medullary, successive, included phloem), leaf mesophyll (dorsiventral), Kranz anatomy (atriplcoid type), trichomes, druses and sandy crystals, C_4 photosynthetic pathway and epidermal characteristics.

Key words: Amaranthaceae, Anatomy, Chenopodiaceae, Lamina, Micro-characters, Stem.

Introduction

The Chenopodiaceae Vent. and Amaranthaceae Juss. are two closely related families of Caryophyllales (Dahlgren, 1980; Cronquist, 1988; Thorne, 1992; Takhtajan, 2009; Kadereit *et al*., 2003 and Reveal, 2012). Recently, it has been proposed to merge both families in Amaranthaceae *s.l.* (APG III and IV, 2009 & 2016; Judd *et al*., 2002) and referred to Chen-Am alliance (Pratt, 2003).

The Amaranthaceae *s.l.* is of worldwide distribution, comprising nearly 169 genera and 2040 species (Christenhusz & Byng, 2016). According to Stevens 2001 onwards, Amaranthaceae *s.l.* comprises five subfamilies *viz.*: Amaranthoideae, Polycnemoideae, Betoideae, Salicornoideae and Chenopodoideae. Amaranthaceae *s.s* are classified into three subfamilies: Amaranthoideae, Polycnemoideae and Gomphrenoideae while Chenopodiaceae are classified to four distinct subfamilies: Chenopodioideae, Salsoloideae, Salicornioideae and Microteoidea (Takhtajan, 2009). Members of these families are dominant in arid, saline, and temperate regions. They are morphologically and anatomically similar and also show C_4 photosynthetic pathway (Borsch *et al*., 2001).

The stem anatomy of Chenopodiaceae and Amaranthaceae is characterized by unusual anatomical features, such as anomalous secondary growth and occurrence of two or more rings of primary vascular bundles (Gibson, 1994). These features may be useful in discrimination between the two families (Cronquist, 1981; Gibson & Nobel, 1986). Fahn & Zimmermann (1982) reported the abnormal secondary growth occurrence in *Atriplex halimus* (successive layers) when a new cambium appears outside the primary vascular bundles in the pericycle. Heklau *et al*., (2012) studied the wood anatomy of Chenopodiaceae (Amaranthaceae *s. l.*) and explained different growth aspects occurred *viz*., successive cambia, included phloem and medullary bundles. De Bary (1884) reported that in the abnormal secondary growth the cambium arose from parenchyma cells between the primary vascular bundle and remained active during the plant life (in Amaranthaceae). Wilson (1924) thought the permanent renewal of cambium. Mao (1933) reported that the medullary bundles of the family formed from a permanent active cambium, while Schinz (1925), believed that the secondary vascular tissue in the stem arise from successive cambia restricted activity.

Kishore (2002) recorded a rayless xylem due to a short life cycle in some studied taxa of Amaranthaceae. Duarte & Debur (2004) found that the most obvious anatomical characteristics of the stem was continuous siphonostelic structure, vascular tissue of only vertical system and two to six vascular bundles in a bipolar position in pith in *Alternanthera brasiliana*. Ravindra *et al.*, (2019) studied the secondary xylem in some taxa and reported that stem sections showed the renewal cambium by replacing with new segments and the secondary phloem formed by earlier cambial segments form isolated islands of phloem, distributed within conjunctive tissues, embedded in the secondary xylem, as the stem grew, a complete ring of cambium was formed.

In Amaranthaceae the dorsiventral mesophyll is the most common while Chenopodiaceae exhibit dorsiventral to centric mesophyll or homogenous rounded cells (Metcalfe & Chalk, 1950).

Chenopodiaceae have the highest number of C_4 species, succulent leaves which are drought tolerant with great diversity in leaf anatomy. The C_4 type of leaves vary in structure, arrangement of chlorenchyma, arrangement of water storage cells, vascular tissue, presence, or absence of various specialized hypodermal cells (Carolin *et al*., 1975).

In Chenopodiaceae, the leaf structure is of two main types; non Kranz and Kranz type (depending on layers of chlorenchyma and vascular bundle embedded in water storage cells). Carolin (1983); Freitag & Stichler (2000 & 2002) and Kadereit *et al*., (2003) recorded 6 types of Kranz anatomy *viz*. Atriplicoid, Kochioid, Salsoloid, Salsinoid, Schoberioid or Kranz-Tecticornoid (Chenopodiaceae). Butnik *et al.*, (2017) reported 14 leaf mesophyll types of kranz anatomy, separating them according to the presence of water bearing cells and spongy parenchyma (Chenopodiaceae and Amaranthaceae).

Metcalfe & Chalk (1950) described various types of hairs (vesicular in Chenopodiaceae or candelabra in *Aerva*; Amaranthaceae) and stomata (anomocytic and anisocytic) on ab- and adaxial leaf surface. Carolin (1983) used leaf trichomes and other morpho-anatomical characters to create an evolutionary suggestion for the Chen-Am alliance. El Ghazali *et al.*, (2016) studied leaf surface of 9 species in Chenopodiaceae and reported sunken, slightly depressed, leveled or slightly raised stomata.

Fank-de-Carvalho *et al*., (2010) reported that the leaf surfaces had epicuticular wax, uniseriate epidermis, amphistomatic leaves, anomocytic stomata in some *Gomphrena* spp. (Amaranthaceae). Members of Chenopodiaceae have small platelets with parallel orientation not restricted to the cells near stomata (Engel & Barthlot, 1988).

Ogundipe & Kadiri (2012) found that the epidermis had curved anticlinal wall on both surfaces, straight anticlinal wall on adaxial surface, anomocytic or paracytic stomata in *Alternanthera* and unicellular, multicellular, candelabra, filiform or peltate trichome of some species in Amaranthaceae.

The specific objective of the present work was to study, investigate and collect the diagnostic stem and lamina anatomical characteristics as well as epidermal micro-characters of the studied taxa. Discus, analyze and evaluate whether these characters can provide additional contribution to the understanding of the closure relationship between Chenopodiaceae and Amaranthaceae.

Material and Methods

Sampling: In the present study, 35 taxa were collected from different natural habitats and botanical gardens in Egypt. Of these, 29 taxa belong to Chenopodiaceae (representing 14 genera, 26 species and three subspecies) and six taxa to Amaranthaceae (representing four genera and six species). The identification of the 30 wild taxa was done with the help of Täckholm (1974) and Boulos (1999), while the cultivated taxa were identified with the aid of Bailey (1949). Synonyms were derived from International Plant Names Index (IPNI; http://www.ipni.org/ipni/plantnamesearchpage.do) and The Plant List (http://www.theplantlist.org/) as shown in Table 1.

Micro-morphological characteristics

Stem and lamina anatomical investigation: Segments from stem and lamina (at third internode) were taken and preserved in F.A.A.sloution (Formalin. Glacial acetic acid. Alcohol). Cross section of the stem and vertical section of lamina were prepared using hand microtome at 10-16 µm, double stained using a combination of safranine and light green then mounted by Canada Balsam according to the customary method of Johansen (1940). The sections were examined using light microscope and photographed by Canon G15digital camera. The internal structures were described with the help of Eames (1929) and Metcalfe & Chalk (1950). Butnik *et al.*, (2017) was followed for Kranz anatomy terminology and description.

Epidermal characteristics: It was carried out for stem peels (in six studied taxa with rudimentary leaves belonging to Chenopodiaceae) and lamina epidermal peels (Stace 1984). The photomicrographs were taken using Canon power- shot G15, 12.1 mega pixels. For SEM investigation, small portions of fresh or dried leaves were fixed on a stub with a double-sided adhesive tape then coated with gold in sputter coater (SPI-Module). Both abaxial- and adaxial surfaces of the lamina was examined and photographed by SEM (JEOL-JSM-5500LV) using high vacuum mode at the Regional Center of Mycology and Biotechnology, Al-Azhar University, Cairo, Egypt.

Results and Discussion

The stem, lamina anatomy as well as epidermal characteristics using LM and SEM of the investigated taxa are presented in Tables 2, 3 & 4. Some of the specific structures (micro-photographs) are illustrated in Figs. 1, 2 & 3 in order to simplify deducing the most diagnostic characters.

Stem anatomical characteristics: The stem outline was triangular in *Beta vulgaris* subsp. *cicla* and *B. vulgaris* subsp. *Maritima* and terete in 5 studied taxa, ridged and furrowed in 10 taxa or ± terete in the remaining 18 taxa (Table 2). Trichomes were e-glandular, branched, candelabra in *Aerva javanica*, e-glandular, multicellular, uniseriate in *Alternanthera dentata*, unicellular glandular and e-glandular in *Amaranthus caudatus*, unicellular, glandular in *Celosia spictata* all belonging to Amaranthaceae. In Chenopodiaceous taxa glandular vesicular trichomes were recorded in *Atriplex halimus*, *A. leucoclada*, *A. lindleyi* subsp. *inflata*, *A. nummularia*, *A. semibaccata*, *Chenopodium opulifolium* and *C. quinoa*, unicellular and multicellular, glandular, and e-glandular in *Bassia arabica* and *Halopeplis amplexicaulis*, glandular vesicular and unicellular in *Salsola inermis*, glandular unicellular in *Salsola volkensii*, e-glandular unicellular and multicellular in *Bassia eriophora*, *B. indica* and *Salsola kali*, or wanting in the remaining 17 studied taxa (Chenopodiaceae and Amaranthaceae). This finding was in accord with Metcalfe & Chalk (1950) who recorded a diverse type of trichomes varying between uniseriate and vesicular in Chenopodiaceae and special types with restricted occurrence in Amaranthaceae. Cuticle was thick in 9 studied taxa or thin in the remaining 26 taxa (Table 2). The multiseriate epidermis was recorded in *Anabasis articulata* and *Haloxylon salicornicum* and the uniseriate epidermis was present in the remaining 33 studied taxa. The epidermis was tangentially- radially elongated in *Atriplex halimus*, *A. lindleyi* subsp. *inflata* and *A. semibaccata*, it was tangentially elongated in 13 studied taxa or radially in 19 taxa. The cortex of collenchyma and parenchyma in 6 studied taxa was palisade, spongy and polyhedral parenchyma (*Arthrocnemum macrostachyum*), chlorenchyma, collenchyma and polyhedral parenchyma (in 5 taxa), collenchyma, parenchyma and sclerenchyma (in 6 taxa), palisade, spongy, parenchyma and sclerenchyma (*Anabasis articulata*, *Halocnemum strobilaceum*, *Haloxylon salicornicum*, *Sarcocornia fruticosa* and *S. perennis*) or collenchyma, chlorenchyma, parenchyma and sclerenchyma (in 12 taxa). The 6 studied taxa with rudimentary leaves and articulated stem belonging to Chenopodiaceae (as assimilating shoots) were characterized by well-developed cortex and narrow pith (Fig. 1; M, N & O), which was in accord with previous studies (Zhibin & Zhang, 2011; Saad Eddin & Doddem, 1986).

Sandy crystals were found in *Beta vulgaris* subsp. *cicla*, *B. vulgaris* subsp. *maritima* and *Amaranthus lividus*, druses & sandy in *Amaranthus caudatus*, *Salsola kali* and *Traganum nudatum*, druses in 15 taxa or wanting in the remaining 14 studied taxa. The type and occurrence of crystals in the present study was in agreement with Grigore *et al.*, (2014) who reported the presence of calcium oxalate crystals in many halophytic Chenopods.

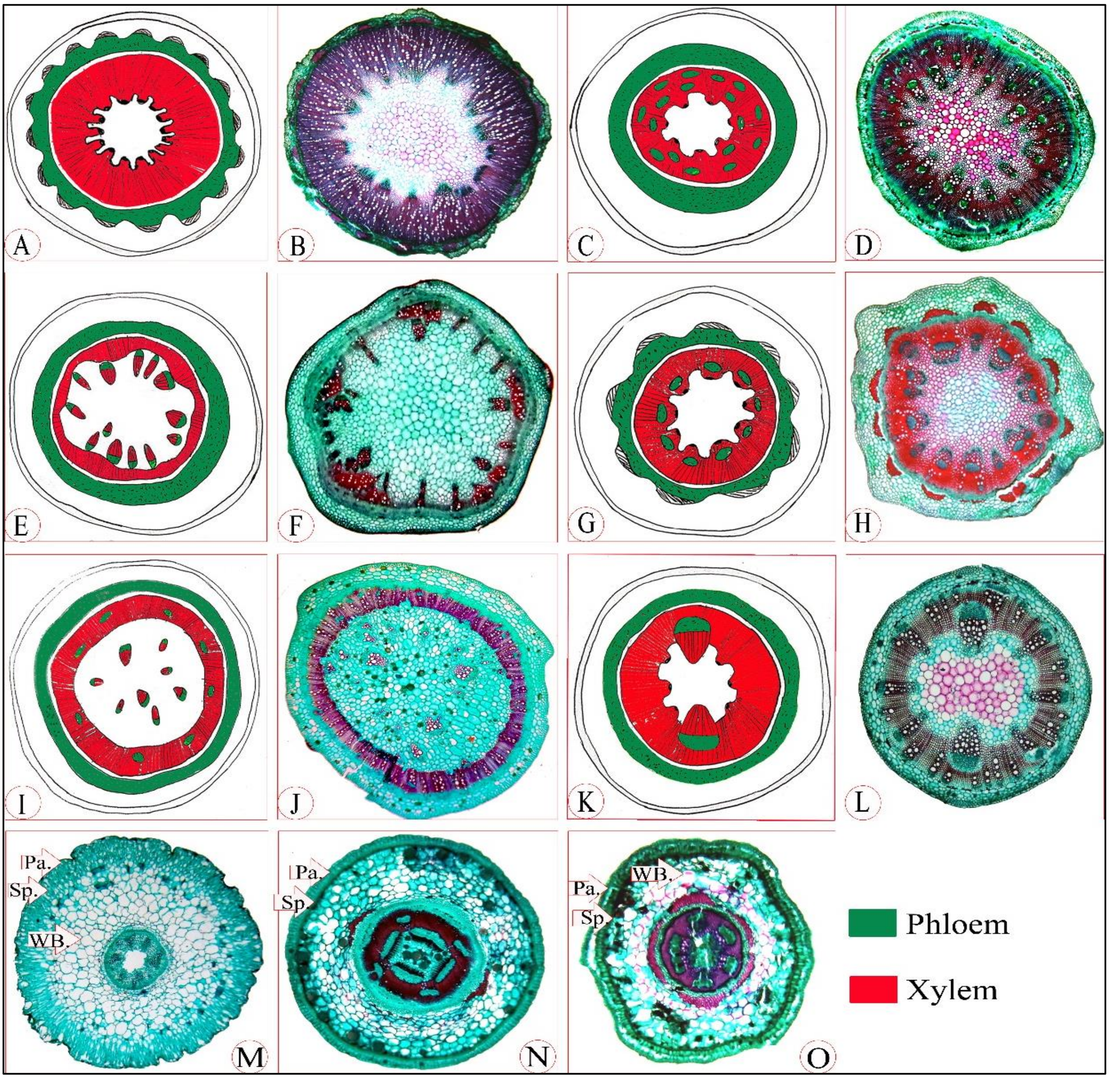

Fig. 1. **(A- L)** Hand drawings and micro-photographs showing different growth aspects of the stem of the studied taxa. **(A, B)** ***Bassia eriophora*;** Normal secondary growth. **(C, D)** ***Atriplex leucoclada*;** Abnormal secondary growth with successive cambium rings. **(E, F)** ***Chenopodium album*;** Abnormal secondary growth with medullary bundles. **(G, H)** ***Suaeda pruinosa*;** Abnormal secondary growth with included phloem. **(I, J)** ***Alternanthera dentata*;** Abnormal secondary growth included phloem & bipolar bundles. **(K, L)** ***Amaranthus lividus*;** Abnormal secondary growth with included phloem & medullary bundles. **(M- O)** micro-photographs showing different growth aspects of the assimilating stem. **(M)** ***Halocnemum strobilaceum***, **(N)** ***Anabasis articulata***, **(O)** ***Haloxylon salicornicum***. (**Pa.)**: Palisade tissue, (**Sp.)**: Spongy tissue, (**WB.)**: Water bearing cell.

Table 1. List of plant taxa and their collection localities in Egypt.

No.		Taxa	Locality/ Source
1.	**Chenopodiaceae**	***Anabasis articulata*** (Forssk.) Moq. = *Salsola articulata* Forssk.	El Alamein, Wadi El- natrun road, Mediterranean coastal region
2.		***Arthrocnemum macrostachyum*** (Moric.) K. Koch = *Salicornia macrostachya* Moric.	Borg El-Arab, Mediterranean coastal region
3.		***Atriplex halimus*** L.	Marriott lake**,** Mediterranean coastal region
4.		***A. leucoclada*** Boiss.	//
5.		***A. lindleyi* subsp. *inflata*** (F.Muell) P. G. Wilson =*Blakiella inflata* (F.Muell.) Aellen	Borg El-Arab, Mediterranean coastal region
6.		***A. nummularia*** Lindl.	Mazhar Botanical garden**,** Cairo
7.		***A. semibaccata*** R. Br.	Marriott lake**,** Mediterranean coastal region
8.		***Bassia arabica*** (Boiss.) Maire & Weiller =*Chenolea arabica* Boiss.	Wadi Habis, Marsa Matruh, Mediterranean coastal region
9.		***B. eriophora*** (Schrad.) Asch. =*Kochia eriophora* Schrad.	Marriott lake**,** Mediterranean coastal region
10.		***B. indica*** (Wight) A.J. Scott =*Kochia indica* Wight	//
11.		***Beta vulgaris* subsp. *cicla*** (L.) W. D. J. Koch *	Botanical garden, Faculty of Science Ain Shams University
12.		***B. vulgaris* subsp. *maritima*** (L.) Thell. =*Beta maritima* L.	Borg El-Arab, Mediterranean coastal region
13.		***Chenopodium album*** L.	Marsa Matruh, Mediterranean coastal region
14.		***C. murale*** L.	//
15.		***C. opulifolium*** Schrad. ex W. D. J. Koch& Ziz	Borg El-Arab, Mediterranean coastal region
16.		***C. quinoa*** Willd.	Agriculture Research Center, Cairo
17.		***Halocnemum strobilaceum*** (Pall.) M. Bieb. =*Salicornia strobilacea* Pall.,	Borg El-Arab, Mediterranean coastal region
18.		***Halopeplis amplexicaulis*** (Vahl) Ung.-Sternb. ex Ces., Pass.& Gibelli = *Salicornia amplexicaulis* Vahl	Wadi Habis, Marsa Matruh, Mediterranean coastal region
19.		***Haloxylon salicornicum*** (Moq.) Bunge ex Boiss. =*Haloxylon schweinfurthii* Asch. = *Hammada elegans* Botsch.	El Salam road**,** Cairo
20.		***Salsola inermis*** Forssk. = *Bassia pulverulenta* H. Lindb. = *Caroxylon inermis* (Forssk.) Akhani& Roalson	Borg El-Arab, Mediterranean coastal region
21.		***S. kali*** L.	Marriott lake**,** Mediterranean coastal region
22.		***S. volkensii*** Schweinf. & Asch. = *Caroxylon volkensii* (Schweinf. & Asch.) Akhani& Roalson	//
23.		***Sarcocornia fruticosa*** (L.) A. J. Scott = *Arthrocnemum fruticosum* (L.) Moq. = *Salicornia fruticosa* (L.) L.	Borg El-Arab, Mediterranean coastal region
24.		***S. perennis*** (Mill.) A. J. Scott = *Salicornia perennis* Mill. = *Arthrocnemum perenne* (Mill) Moss	//
25.		***Spinacia oleracea*** L. * = *Spinacia glabra* Mill.	Botanical garden, Faculty of Science, Ain Shams University
26.		***Suaeda maritima*** (L.) Dumort. = *Suaeda indica* (Willd) Moq.	Borg El-Arab, Mediterranean coastal region
27.		***S. pruinosa*** Lange	Marsa Matruh, Mediterranean coastal region
28.		***S. vera*** Forssk. ex J.F. Gmel. = *Salsola fruticosa* (L.) L., = *Suaeda fruticosa* (L.) Dumort.	Borg El-Arab, Mediterranean coastal region
29.		***Traganum nudatum*** Delile	Wadi Habis, Mediterranean coastal region
30.	**Amaranthaceae**	***Aerva javanica*** (Burm. f.) Juss. Ex. Schult. = *Iresine javanica* Burm. f.	Nuweibaa, Taba road**,** South Sinai
31.		***Alternanthera dentata*** Scheygrond *	Botanical garden, Faculty of Science, Ain Shams University
32.		***Amaranthus caudatus*** L.	//
33.		***A. lividus*** L.	//
34.		***Celosia argentea*** L. *	El Orman botanical garden, Cairo
35.		***C. spicata*** Spreng. *	//

(=): synonym, (//): as previous, (*): cultivated species

Table 2. Stem anatomical characteristics of the taxa studied under light microscope

	Outline	Trichomes	Cuticle	Epidermis		Cortex	Pith		Growth aspect	Crystals	Fascicular & inter-fascicular xylem components
				Rows	Shape		Width	Cells			
1.	Terete	Absent	Thin	Multiseriate	Tangential	Pa., Sp., Par., Scl.	Narrow	Thin walled	Abnormal Included phloem	Druses	Same
2.	± Terete	//	//	Uniseriate	Radial	Pa., Sp., Par.	//	//	//	Absent	Different
3.	Ridged & furrowed	Glandular vesicular	//	//	Tangential/ radial	Col., Chl., Par.	Wide	//	Abnormal successive cambia	Druses	Same
4.	± Terete	//	//	//	Tangential	Col., Par., Scl.	Narrow	Lignified	//	//	//
5.	Ridged & furrowed	//	//	//	Tangential/ radial	Col., Chl., Par.	Wide	Thin walled	//	//	//
6.	//	//	//	//	Radial	Col., Chl., Par., Scl.	Narrow	Lignified	Abnormal Included phloem	//	Different
7.	//	//	//	//	Tangential/ radial	Col., Chl., Par.	Wide	Thin walled	Abnormal successive cambia	//	Same
8.	//	Unicellular glandular & multicellular e-glandular	//	//	Radial	Col., Par., Scl.	Narrow	//	Normal	Absent	Different
9.	± Terete	e-glandular unicellular & multicellular	Thick	//	//	//	Wide	//	//	//	Same
10.	//	//	//	//	//	//	//	//	//	//	//
11.	Triangular	Absent	//	//	Tangential	Col., Chl., Par., Scl.	//	//	Abnormal medullary bundles	Sand	Different
12.	//	//	//	//	//	//	//	//	Normal	//	//
13.	± Terete	//	//	//	//	//	//	//	Abnormal medullary bundles	Druses	//
14.	Ridged & furrowed	//	//	//	Radial	//	Narrow	//	Normal	//	//
15.	//	Glandular vesicular	//	//	//	//	Wide	//	Abnormal Included phloem	//	//
16.	± Terete	//	//	//	Tangential	//	//	//	Abnormal medullary bundles	//	//
17.	//	Absent	Thin	//	Radial	Pa., Sp., Par., Scl.	Narrow	//	Abnormal Included phloem	Absent	//
18.	//	Unicellular & multicellular glandular & e-glandular	//	//	//	Col., Par., Scl.	//	//	//	Druses	Same
19.	Terete	Absent	//	Multiseriate	Tangential	Pa., Sp., Par., Scl.	//	//	//	//	//
20.	± Terete	Glandular, vesicular & unicellular	//	//	Radial	Col., Par.	Narrow	//	//	Absent	Different
21.	Terete	e-glandular unicellular & multicellular	//	//	//	Col., Chl., Par., Scl.	Wide	//	//	Druses & sand	//
22.	± Terete	Glandular, unicellular	//	//	Tangential	Col., Par.	Narrow	//	//	Absent	Same
23.	//	Absent	//	//	Radial	Pa., Sp., Par., Scl.	//	//	//	//	//
24.	//	//	//	//	//	//	//	//	//	//	//
25.	Terete	//	//	//	//	Col., Par.	Wide	Pith cavity	Abnormal medullary bundles	//	//
26.	± Terete	//	Thick	//	//	Col., Par., Scl.	Narrow	Thin walled	Abnormal Included phloem	//	//
27.	Ridged & furrowed	//	Thin	//	//	Col., Chl., Par., Scl.	Wide	//	//	//	//
28.	//	//	//	//	//	//	Narrow	//	//	//	//
29.	± Terete	Absent	Thick	//	Tangential	Col., Par.	//	//	//	Druses & sandy	//
30.	//	e-glandular candelabra	Thin	//	//	Col., Chl., Par., Scl.	Wide	Lignified& nonlignified	//	Druses	//
31.	//	e-glandular multicellular, uniseriate	//	//	//	//	//	Thin walled	Abnormal Included phloem with bipolar bundle	//	//
32.	Terete	Unicellular glandular &e-glandular	//	//	Radial	Col., Chl., Par.	Narrow	//	Abnormal medullary bundles	Druses & sand	Different
33.	± Terete	Absent	//	//	Tangential	//	Wide	//	Abnormal Included phloem with medullary bundles	Sand	//
34.	Ridged & furrowed	//	//	//	Radial	Col., Par.	//	//	Abnormal successive cambia	Druses	//
35.	± Terete	Unicellular glandular	//	//	Tangential	//	Narrow	//	//	Absent	//

(//): as previous, (±): more or less, (Chl.): isodiametric chlorenchyma, (Col.): angular collenchyma, (Pa.): palisade, (Par.): polyhedral parenchyma, (Scl.): sclerenchyma, (Sp.): spongy

Table 3. Lamina anatomical characters of the taxa studied under light microscope.

	Outline	Trichomes	Epidermis Shape	Mesophyll type	Palisade	Mechanical tissue	Ground tissue	Crystals	Kranz anatomy	Vascular bundles
1.	-	-	-	-	-	-	-	-	-	-
2.	-	-	-	-	-	-	-	-	-	-
3.	Rounded ab- & adaxially	Glandular vesicular	Tangential	Isolateral	One row ab-& adaxially	Angular collenchyma	Parenchyma (WBT)	Druses	Present	Four
4.	Convex ab- & rounded adaxially	//	//	//	//	//	//	//	//	//
5.	Flattened differentiated into midrib & wings	//	//	//	//	Absent	//	//	//	One
6.	Rounded ab- & adaxially	//	//	//	//	Angular collenchyma	//	//	//	Two
7.	Flattened differentiated into midrib & wings	//	//	//	//	Absent	//	//	//	Four
8.	Ovate not differentiated into midrib & wings	Absent	//	Centric	One ring	//	Palisade & parenchyma (WBT)	Sandy	//	14-15
9.	Ribbon like not differentiated into midrib & wings	//	//	//	//	//	//	Druses	//	Three with numerous peripheral
10.	//	//	//	//	//	//	//	//	//	One with numerous peripheral
11.	Wavy ab- & convex adaxially	//	//	Dorsiventral	One row	Angular collenchyma	Polyhedral parenchyma	Druses & sand	Absent	One
12.	Straight ab- & convex adaxially	//	//	Isolateral	//	//	//	//	//	//
13.	Rounded ab- & straight adaxially	//	//	Dorsiventral	One row	Absent	//	Druses	//	Three
14.	Flattened differentiated into midrib & wings	//	//	Isolateral	One row ab-& adaxially	//	//	Absent	//	One
15.	//	Glandular vesicular	//	//	//	//	//	//	//	Three
16.	Rounded ab- & adaxially	//	//	Dorsiventral	One row	//	//	Druses	//	//
17.	-	-	-	-	-	-	-	-	-	-
18.	Triangular not differentiated into midrib & wings	Absent	Radial	Centric	One ring	Absent	Parenchyma (WBT)	Druses	Present	Four with few peripheral

Table 3. (Cont'd.).

	Outline	Trichomes	Epidermis Shape	Mesophyll type	Palisade	Mechanical tissue	Ground tissue	Crystals	Kranz anatomy	Vascular bundles
19.	-	-	-	-	-	-	-	-	-	-
20.	Semi- terete not differentiated into midrib & wings	Glandular vesicular	Tangential	Centric	One row	Absent	Palisade, kranz cells &WBT	Druses	Present	Three with numerous peripheral adaxially
21.	Terete	E-glandular unicellular & multicellular	Radial	//	One ring	//	//	//	//	One with numerous peripheral
22.	Semi- terete not differentiated into midrib & wings	Glandular unicellular	Tangential	//	One row	//	//	//	//	One with numerous peripheral adaxially
23.	-	-	-	-	-	-	-	-	-	-
24.	-	-	-	-	-	-	-	-	-	-
25.	Rounded ab- & straight adaxially	Absent	Tangential	Dorsiventral	One row	Absent	Folded parenchyma	Druses	Absent	Three & two small, winged strands
26.	Ovate not differentiated into midrib & wings	//	//	Centric	One ring	//	Palisade, kranz cells &WBT	//	Present	One large & 8 small
27.	//	//	//	//	//	//	Palisade &WBT	Absent	Absent	One large & 11 small
28.	//	//	//	//	//	//	//	//	//	//
29.	Kidney- shaped not differentiated into midrib & wings	//	//	//	//	//	Palisade, kranz cells &WBT	Druses	Present	One with numerous peripheral
30.	Crescent form	e-glandular candelabra	//	Ill-defined	Absent	//	Folded parenchyma	//	Absent	One
31.	Rounded ab- & adaxially	e-glandular multicellular	//	Dorsiventral	One row	//	Polyhedral parenchyma	//	//	Four
32.	Basin-like	//	Radial	//	//	Angular collenchyma	Folded parenchyma	Sandy	Present	Three
33.	Crescent	Absent	Tangential with bulliform cells	//	//	Absent	Polyhedral parenchyma	Druses& sandy	//	//
34.	Rounded ab- & adaxially	//	Tangential	//	//	//	//	Absent	Absent	One
35.	Wavy ab- & rounded adaxially	//	//	//	//	//	Folded parenchyma	Druses	//	//

(//): as previous, (-): not practically available, (WBT): water bearing tissue

Table 4. Epidermal characteristics of the taxa studied under light microscope and scanning electron microscope.

	Leaf type	LM			SEM			
		Cells shape (Ab-/ Adaxial)	Anticlinal wall (Ab-/ Adaxial)	Stomata type (Ab-/ Adaxial)	Sculpture (Ab-/ Adaxial)	Stomata shape (Ab-/ Adaxial)	Stomata elevation (Ab-/ Adaxial)	Epicuticular wax (Ab-/ Adaxial)
1.	Stem peels	Polygonal/ -	Straight/ -	Brachyparacytic/ -	-	-	-	-
2.	//	//	//	//	-	-	-	-
3.	Amphistomatic	Polygonal/ Same	Straight/ Same	Anomocytic & brachyparacytic/ Same	Ruminate/ Same	Elliptic with slit-like opening/ Same	Sunken/ Same	Platelets & granules/ Same
4.	//	//	//	Anomocytic & brachyparacytic/ Anomocytic	//	Elliptic/ Same	Leveled/ Same	Granules/ Same
5.	//	//	//	Anomocytic/ Same	//	-/-	-/-	Platelets& granules/ platelets
6.	//	//	//	//	//	-/-	-/-	Platelets & granules/ Same
7.	//	//	//	Anomocytic & brachyparacytic/ Same	Ruminate/ Oscillate-reticulate	-/ Elliptic	-/ Sunken	Granules/ platelets& granules
8.	//	//	//	//	Reticulate/ Same	Elliptic/ Same	Sunken/ Same	Rhomboid& platelets/ Same
9.	//	//	//	Brachyparacytic/ Same	Reticulate/ Same	Slit-like/ Same	Leveled/ Same	Platelets & granules/ Same
10.	//	//	//	//	//	//	//	//
11.	//	Irregular/ Same	Sinuate/ Same	Anomocytic/ Same	Ruminate/ Same	Elliptic/ Same	Elevated/ Leveled	Granules/ Same
12.	//	//	//	//	Reticulate/ Same	//	Leveled/ Same	//
13.	//	//	Curved/ Same	Anomocytic/ Same	Ruminate/ Same	//	Elevated/ Sunken	Platelets/ Same
14.	//	Irregular/ Polygonal	Sinuate/ Straight	//	Ruminate/ Ill-defined	//	Leveled/ Sunken	Granules/ Fissured crust & granules
15.	//	Polygonal/ Same	Straight/ Same	Anomocytic & Brachyparacytic/ Same	Rugose/ Same	Slit-like/ Same	Sunken/ Leveled	Platelets & granules/ Same
16.	//	Polygonal/ Same	Straight/ Same	Anomocytic/ Same	Reticulate/ Same	Elliptic/ Same	Sunken/ Same	Platelets & granules/ Same
17.	Stem peels	Polygonal/-	Straight/ -	Brachyparacytic/ -	-	-	-	-
18.	Amphistomatic	Polygonal/ Same	Straight/ Same	Brachyparacytic/ Same	Rugose/ Ruminate	Slit-like/ Elliptic	Sunken/ Leveled	Granules/ Same
19.	Stem peels	Polygonal/-	Straight/ -	Brachyparacytic/ -	-	-	-	-
20.	Hypostomatic	Polygonal/ Same	Straight/ Same	//	Reticulate/ Rugose	Oval/ -	Leveled/-	Granules/ Same
21.	Amphistomatic	//	//	Brachyparacytic/Anomocytic & brachyparacytic	Colliculate/ Same	Elliptic/ Slit-like	Leveled/ Same	//
22.	Hypostomatic	//	//	Brachyparacytic/ -	Colliculate/ Rugose	-	-	//
23.	Stem peels	Polygonal/-	Straight/ -	//	-	-	-	-
24.	//	//	//	//	-	-	-	-
25.	Amphistomatic	Irregular/ Polygonal	Sinuate/ Straight	Anomocytic/ Same	Ruminate/ Same	Elliptic/ Same	Elevated/ Same	Granules/ Same
26.	//	Polygonal/ Same	Straight/ Same	Brachyparacytic/ Same	Rugose/ Same	Slit-like/ Same	Leveled/ Same	Crust& granules/ Platelets, fissured crust & granules
27.	//	//	//	//	Ill-defined/ Same	//	//	Platelets/ Same
28.	//	//	//	//	Rugose/Same	-/ Elliptic	-/ Sunken	-/ -
29.	//	//	//	//	Reticulate/ Same	Elliptic/ Same	Sunken/ Leveled	Platelets/ Granules
30.	//	Irregular/ Polygonal	Sinuate/ Straight	Anomocytic/ Brachyparacytic	Colliculate/ Reticulate	//	Leveled/ Same	Granules/ Same
31.	//	//	//	Anomocytic/ Same	Rugose/ Same	//	//	//
32.	//	//	//	//	Ruminate/ Same	//	Elevated/ Same	//
33.	//	Irregular/ Same	Sinuate/ Same	//	Rugose/ Ruminate	//	//	//
34.	//	Irregular/ Polygonal	//	//	Ruminate/ Same	//	Leveled/ Sunken	//
35.	//	Irregular/ Same	Undulate/ Same	//	//	Slit-like/ Same	Leveled/ Same	//

(//): as previous, (-): not practically available

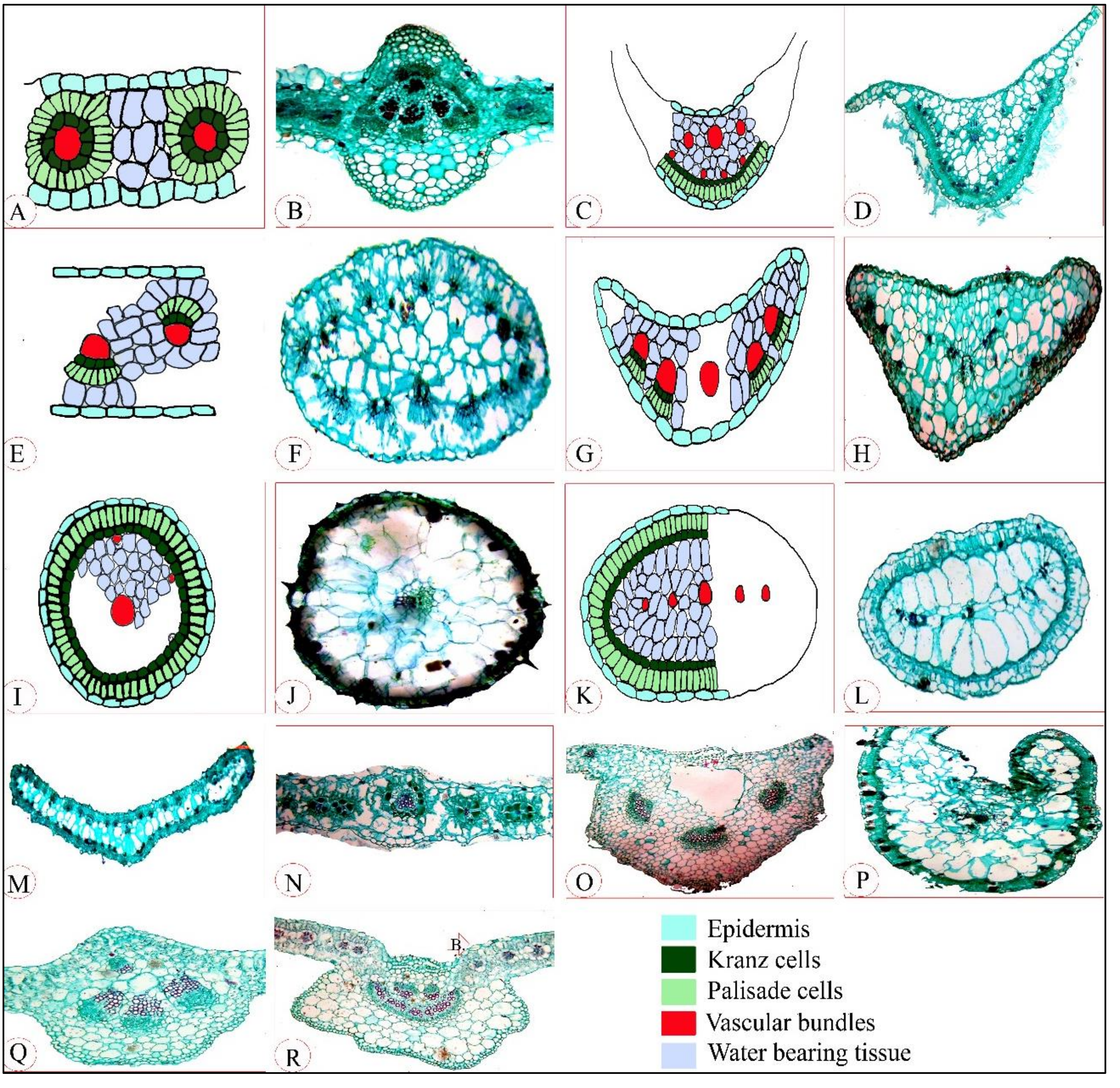

Fig. 2. **(A- L)** Hand drawing and microphotographs of lamina anatomical characteristics showing different types of Kranz anatomy, outlines shapes & vascular supplies. **(A, B)** ***Atriplex leucoclada*;** Atriplicoid type, **(C, D)** ***Salsola inermis*;** Flat-leaved salsoloid type. **(E, F)** ***Bassia arabica*;** Kochoid type, **(G, H)** ***Halopeplis amplexicaulis*;** Kranz-ventrodorsal type **(I, J)** ***Salsola kali*;** Salsoloid type. **(K, L)** ***Suaeda maritima*;** Salsina type. **(M-R);** Major aspects of lamina anatomical characteristics showing different outlines shapes & vascular supplies. **(M)** ***Bassia indica*, (N)** ***Atriplex lindleyi*** **subsp** ***inflate*****, (O)** ***Spinacia oleracea*****, (P)** ***Traganum nudatum*****, (Q)** ***Alternanthera dentate*****, (R)** ***Amaranthus caudatus***. (**B.**): Bulliform cell.

Two major types of secondary growth aspects were recorded *viz.* normal secondary growth in *Bassia arabica*, *B. eriphora*, *B. indica*, *Beta vulgaris* subsp. *maritima* and *Chenopodium murale* (Fig. 1, A & B) and abnormal or anomalous secondary growth in the remaining 30 studied taxa. The latter aspect (abnormal) can be categorized into five sub-types *viz.* successive cambia in *Atriplex halimus*, *A. leucoclada*, *A. lindleyi* subsp. *inflata*, *A. semibaccata*, *Celosia argentea & C. spicata* (Fig. 1, C & D), medullary bundles in *Beta vulgaris* subsp. *cicla*, *Chenopodium album*, *C. quinoa*, *Spinacia oleracea & Amaranthus caudatus* (Fig. 1, E & F), included phloem in 17 taxa (Fig. 1, G & H), included phloem and bipolar vascular bundles in *Alternanthera dentata* (Fig. 1, I & J) or included phloem and medullary bundles in *Alternanthera dentata* and *Amaranthus lividus* (Fig. 1, K, L).

The foregoing stem anatomical data is in accord with Esau (1965) who reported the occurrence of medullary bundles as an aspect of anomalous secondary growth. Metcalfe & Chalk (1950) reported that abnormal secondary growth occurred in all species of Chenopodiaceae that had relatively thick stems in contrast with genera of thin stems. Chandurkar (1983) reported the presence of vascular bundles in the pith in addition to the normal ring of vascular bundles, and treated them as ‘leaf trace bundles’, due to the entering of leaf traces to the node and running through internodes before reaching the vascular system in the stem. Costea & Demason (2001) reported the same mechanisms of secondary growth formation in Chenopodiaceae and Amaranthaceae. Heklau *et al*., (2012) reported the anomalous secondary thickening was widespread in Amaranthaceae and Chenopodiaceae as well as the successive cambia and included secondary phloem were typical in Chenopodiaceae. The inter-fascicular xylem regions were filled with xylem vessels, xylem fibers & xylem parenchyma in 19 studied taxa or sclerenchymatous tissue in the remaining 16 studied taxa.

The pith was wide in 17 taxa or narrow in the remaining 18 taxa. Lignified pith parenchyma was recorded in *Atriplex leucoclada* and *A. nummularia*. Lignified and non-lignified parenchyma was in *Aerva javanica* and *Spinacia oleracea.* The pith cavity or thin-walled parenchyma was reported in the remaining 31 studied taxa. This finding agreed with (Zhibin & Zhang**,** 2011; Saad Eddin & Doddem, 1986).

Lamina anatomical characteristics: The lamina outline was terete in *Salsola kali*, triangular, not differentiated into mid-vein and wings in *Halopeplis amplexicaulis,* kidney-shaped not differentiated into mid-vein and wings in *Traganum nudatum,* wavy abaxially- convex adaxially in *Beta vulgaris* subsp. *cicla,* straight abaxially- convex adaxially in *Beta vulgaris* subsp. *maritima,* wavy abaxially- rounded adaxially in *Celosia spictata,* basin-like in *Amaranthus caudatus,* convex abaxially- rounded adaxially in *Atriplex leucoclada,* ribbon-like not differentiated into mid-vein and wings in *Bassia eriophora* and *B. indica,* semi-terete not differentiated into mid-vein and wings in *Salsola inermis* and *S. volkensii*, rounded abaxially- straight adaxially in *Chenopodium album* and *Spinacia oleracea,* ovate not differentiated into mid-vein and wings in *Bassia arabica*, *Suaeda maritima*, *S. pruinosa* and *S. vera,* crescent form in *Aerva javanica* and *Amaranthus lividus*, rounded abaxially- and adaxially in 5 taxa or flattened not differentiated into mid-vein and wings in the remaining 4 studied taxa (Table 3; Fig. 2).

The epidermal cells were tangentially elongated with bulliform cells in *Amaranthus lividus*, radial in *Halopeplis amplexicaulis*, *Salsola kali* and *Amaranthus caudatus* or tangential in the remaining 25 studied taxa.

Trichomes were candelabra in *Aerva javanica*, multicellular in *Alternanthera dentata* and *Amaranthus caudatus* belong to Amarantaceae, e-glandular unicellular and multicellular in *Salsola kali*, glandular unicellular in *S. volkensii*, vesicular in eight studied taxa belong to Chenopodiaceae or wanting in the remaining 16 studied taxa. This was in accord with Batanouny (2001) who reported the occurrence of salt bladders in all species of *Atriplex* and some species of *Salsola* and *Chenopodium*. Lu *et al*., (2012) also detected salt bladders on the leaves of *Chenopodium album*. El Ghazali *et al*., (2016) reported a diversity in trichomes types in *Salsola* sp.

The ground tissue in the form of folded parenchyma in 5 taxa, water bearing tissue (WBT) in 5 taxa, palisade and WBT in 6 taxa, Palisade and WBT with Kranz cells in 5 taxa or polyhedral parenchyma in 8 studied taxa. Mechanical tissue in the form of angular collenchyma abaxially- and adaxially at mid-vein was recorded in 6 taxa (Chenopodiaceae and Amaranthaceae) or wanting in the remaining 23 studied taxa. Nine out of 29 studied taxa of Chenopodiaceae was characterized morphologically by fleshy leaves having water bearing cells anatomically.

The mesophyll was ill-defined in *Aerva javanica* (Amraranthaceae) dorsiventral in *Beta vulgaris* subsp. *cicla*, *Chenopodium album*, *C. quinoa*, *Spinacia oleracea* of Chenopodiaceae, *Alternanthera dentata*, *Amaranthus caudatus*, *A. lividus*, *Celosia argentea* & *C. spicata* of Amranthaceae, isolateral in *Atriplex halimus*, *A. leucoclada*, *A. lindleyi* subsp. *inflata*, *A. semibaccata*, *Beta vulgaris* subsp. *maritima*, *Chenopodium murale* and *C. opulifolium* of Chenopodiaceae or centric in the remaining 11 taxa (Chenopodiaceae). The foregoing data was in line with Metcalfe & Chalk (1950) who reported that, the leaves of Chenopodiaceae exhibited dorsiventral to centric or composed of homogenous rounded cells while in Amaranthaceae dorsiventral mesophyll was the most common but isolateral was also recorded in few species.

Number of vascular bundles were 2 in *Atriplex nummularia*, 14-15 in *Bassia arabica*, one large and 8 small in *Suaeda maritima*, one large and 11 small in *S. pruinosa* and *S. vera*, 4 in 5 taxa, 3 in 8 taxa or one bundle in the remaining 11 studied taxa. Sand crystals and druses were present in 30 taxa or absent in five taxa.

Out of 35 studied taxa, two main types of leaf structure were recorded; Kranz type (16 studied taxa) and non Kranz type (13 studied taxa). The data concerning lamina micro-characters 6 kranz types were recognized (based on type of leaf mesophyll, size, and distribution of kranz), **i. atriplcoid type** in *Atriplex halimus*, *A. leucoclada*, *A. lindleyi* subsp. *inflata*, *A. nummularia*, *A. semibaccata*, *Amaranthus caudatus* and *A. lividus* (Amaranthaceae and Chenopodiaceae; Fig. 2, A& B), **ii. flat- leaved salsoloid type** in *Salsola inermis* and *S. volkensii* (Chenopodiaceae; Fig. 2, C& D), **iii. kochioid type** in *Bassia arabica*, *B. eriophora* and *B. indica* (Chenopodiaceae; Fig. 2, E& F), **iv. kranz-ventrodorsal** in *Halopeplis amplexicaulis* (Chenopodiaceae; Fig. 2, G& H), **v. salsoloid type** in *Salsola kali* and *Traganum nudatum* (Chenopodiaceae; Fig. 2, I& J), **vi. salsina type** in *Suaeda maritima* (Chenopodiaceae; Fig. 2, K& L). The non-kranz or Axiroid type was detected in the rest 13 taxa belonging to Amaranthaceae and Chenopodiaceae (Fig. 2, O). This is in accord with Butnik *et al*., (2017), they registered 14 leaf types based on mesophyll, kranz cell occurrence. Hauri (1912), Moser (1934) and Rosengart-Famel (1937) examined the distribution of Kranz anatomy in *Atriplex* and provided good quality data on the anatomy and distribution of the Kranz syndrome in other genera. Metcalfe & Chalk (1950), Napp-Zinn (1973), Voznesenskaya *et al*., (2001& 2002) reported that species in the Chenopodiaceae had unusual chlorenchyma and Kranz anatomy.

Chenopodiaceae contained the highest number of C_4 species and C_4 lineages among eudicots (Kadereit *et al*., 2003, 2012; Sage *et al*., 2011). The studied taxa were divided into two groups; 16 taxa with kranz anatomy having C_4 photosynthetic pathway and the remaining 13 taxa with non kranz anatomy having C_3 photosynthetic pathway and this data was in accord with Freitag & Kadereit (2014).

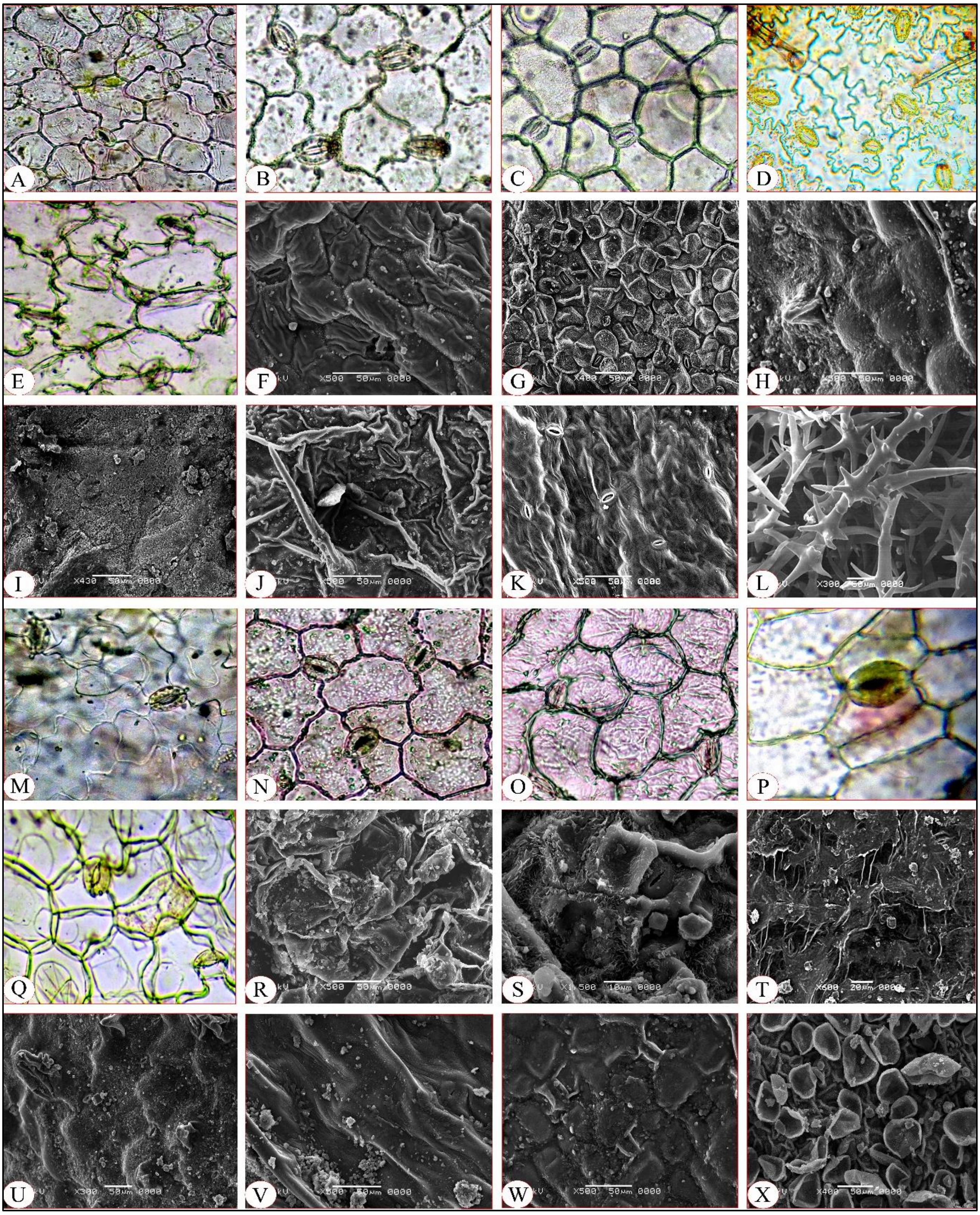

Fig. 3. **A- L**. Major aspects of lamina epidermis (LM & SEM; abaxial surface). **(A)** ***Beta vulgaris* subsp. *Maritime***, **(B)** ***Chenopodium album,*** **(C)** ***Salsola kali***, **(D)** ***Alternanthera dentate,*** **(E)** ***Celosia spicata,*** **(F)** ***Bassia arabica;*** Reticulate sculpture, platelets wax **(G)** ***Chenopodium quinoa;*** Reticulate sculpture, platelets & granulate wax, **(H)** ***Salsola kali;*** Colliculate sculpture, granulate wax. **(I)** ***Suaeda pruinosa;*** platelets wax, **(J)** ***Alternanthera dentata;*** Rugose sculpture, granulate wax, **(K)** ***Amaranthus lividus;*** Ruminate sculpture, granulate wax, **(L)** ***Aerva javanica;*** Candelabra trichomes. **(M- X);** Major aspects of lamina epidermis (LM & SEM; adaxial surface). **(M)** ***Beta vulgaris* subsp. *Cicla,*** **(N)** ***Beta vulgaris* subsp. *maritima,*** **(O)** ***Chenopodium album,*** **(P)** ***Suaeda pruinosa***, **(Q)** ***Celosia spicata,*** **(R)** ***Atriplex halimus;*** Ruminate sculpture, plateltes & granulate wax. **(S)** ***Atriplex semibaccata;*** Oscillate-reticulate sculpture, platelets & granulate wax. **(T)** ***Chenopodium murale;*** Fissured crust & granulate wax. **(U)** ***Salsola kali;*** Colliculate sculpture, granulate wax. **(V)** ***Suaeda maritima;*** Rugose sculpture, granulate, platelets & fissured crust wax. **(W)** ***Traganum nudatum;*** Reticulate sculpture, granulate wax. **(X)** ***Chenopodium opulifolium;*** Vesicular trichomes.

The six studied taxa with rudimentary leaves belong to Chenopodiaceae having stem with anatomical leaf-like structure were considered C_4 plants as kranz anatomy was detected in these assimilatory shoots and this finding was in accord with Saad Eddin & Doddem (1986) and Zhibin & Zhang (2011).

Epidermal characteristics: (LM), in stem peels of the six studied taxa with rudimentary leaves, the stomata were brachyparacytic. In a typical lamina, the leaf was hypostomatic in *Salsola inermis* and *S. volkensii* or amphistomatic in the remaining studied taxa. Based on stomatal variation the leaf type was heterostomatic (brachyparacytic and anomocytic) in *Atriplex halimus*, *A. leucoclada*, *A. semibaccata*, *Bassia arabica*, *Chenopodium opulifolium* and *S. kali* belon to Chenopodiaceae or homostomatic (anomocytic or brachyparacytic) in the remaining 23 studied taxa. Metcalfe & Chalk, (1950) registered the ranunculaceous stomata in the majority of Chenopodiaceae and rubiaceous in few genera. Also, they reported that Amaranthaceae had amphistomatic leaves. Abaxial cell shape was irregular in 11 taxa or polygonal in the remaining 18 taxa. Abaxial anticlinal wall ranged from sinuate in two taxa, curved in *Chenopodium album*, sinus in five taxa, undulate in *Celosia spicata* or straight in the rest 24 studied taxa (Fig. 3, A- E). Adaxial cell shape was irregular in 5 taxa or polygonal in the remaining 24 taxa. Adaxial anticlinal wall was curved in *Chenopodium album*, sinus in *Beta vulgaris* var. *cicla*, sinuate in *Beta vulgaris* subsp. *maritima*, *Amaranthus lividus*, undulate in *Celosia spictata* or straight in the remaining 24 studied taxa (Fig. 3, M- Q).

Four patterns of abaxial epidermal surface sculpture were recorded *viz.* reticulate, colliculate, rugose or ruminate (Fig. 3, F- K) and 5 patterns for adaxial surface *viz.* ruminate, oscillate-reticulate, colliculate, rugose or reticulate (Fig. 3, R- W). Four patterns of abaxial epicuticular wax *viz*. platelets, granules, rhomboid crystals, or crust were observed and 4 patterns of adaxial epicuticular wax *viz*. fissured crust, platelets, rhomboid crystals, and granules were recorded. The foregoing data of epidermal surface sculpture and epicuticular wax agreed with Engel & Barthlott (1988) and Jetter & Riederer (1994), who observed different shapes of platelets with various orientations on the leaves of some taxa in Chenopodiaceae.

Ab-/Adaxial stomatal shape: It was elliptic with slit-like opening in *Atriplex halimus*, oval in *Salsola inermis*, slit-like in 7 taxa or elliptic in 15 taxa. From the epidermal characteristics investigation, there was a minor difference between the ab- and adaxial surfaces in Chenopodiaceae and this result coincided with Zarinkamara (2007).

Conclusion

Most of the obtained data of the studied taxa contribute greatly in understanding the relationships between Chenopodiaceae and Amaranthaceae and reinforce the alliance between them. The most common characters that support this alliance are anomalous secondary growth (medullary, successive, included phloem), leaf mesophyll (dorsiventral), Kranz anatomy (atriplcoid type), trichomes, druses and sandy crystals, C_4 photosynthetic pathway and epidermal characteristics. This finding is similar to that of Judd & Ferguson (1999), Judd *et al*., (2002), APG III, IV (2009 & 2016), Stevens (2001 onwards) and Shipunov (2021) who stated that the Chenopodiaceae nested within Amaranthaceae *s.l.* based on molecular criteria. The authors recommended further studies based on morphological, chemical, and molecular investigation for more accurate systematic relationships between the two families.

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