Annals of Agricultural Science (2012) 57(1), 73–79

Faculty of Agriculture, Ain Shams University

Annals of Agricultural Science

www.elsevier.com/locate/aoas

ORIGINAL ARTICLE

Anatomical traits of some species of *Kalanchoe* (Crassulaceae) and their taxonomic value

H.S. Abdel-Raouf *

General Botany Branch, Department of Agricultural Botany, Faculty of Agriculture, Al Azhar University, Nasr City, Cairo, Egypt

Received 1 December 2011; accepted 18 December 2011
Available online 5 April 2012

KEYWORDS

Kalanchoe;
Taxonomy;
Stem anatomy;
Leaf anatomy;
Indented key

Abstract Anatomical studies of the stems and leaves of 15 species of the genus *Kalanchoe* were studied. Anatomical examination of the cross sections of the above mentioned stems and leaves revealed diagnostic characters among species. Data of comparative characters reached 42 couplet characters; data matrix was organized on the basis of variations to obtain a classification using sequential indented key. Data matrix included the anatomical description and features of the epidermis, cortex, pericycle, vascular bundles and pith for stem anatomy and epidermis, mesophyll, midrib region and vascular bundles for leaf anatomy.

Introduction

Kalanchoe genus belongs to Crassulaceae, defined broadly; this genus has about 125 described species, mostly in Africa, Madagascar, Brazil and a few in the tropical area; several are seen in green houses (Bailey, 1951). *Kalanchoe* representing crassulacean acid plants (CAMp.) which are recognized as a photosynthetic pathway distinct from C_3 and C_4. It is known to occur in at least 25 angiosperm families (Ting, 1982; Winter, 1985), as well as a few gymnosperms and ferns species (Warmbrodt, 1984). These plants are termed succulent plants, characterized by thickened stems and leaves modified for water and acid storage (Kluge and Ting, 1978). Mesophyll cells of (CAM) plants are not usually differentiated into palisade and spongy parenchyma (Kluge and Ting, 1978). There tends to be less free air space between mesophyll cells of C_3 and C_4 (Luttge and Ball, 1977). In general, Crassulaceae characterized anatomically by the presence of sandy crystals in some members, leaf structure is as a rule, centric or intermediate between bifacial and centric in structure, palisade tissue is rarely present (Metcalfe and Chalk, 1950). Cortex in stem is usually fleshy and strongly developed, consists of succulent parenchyma, also contains weakly developed collenchymas. The cork is developed in the epidermis or in sub-epidermal cell layer or a deeper layer of the primary cortex becomes the phellogen (Metcalfe and Chalk, 1950). Cortical bundles are present commonly, or absent (Watson and Dallwitz, 1992). The present work aims to verify the anatomical variations between the different studied species from *Kalanchoe* plants to use these anatomical

* Mobile: +20 0100 0430891.
E-mail address: Dr-hany70@hotmail.com

0570-1783 © 2012 Faculty of Agriculture, Ain Shams University. Production and hosting by Elsevier B.V. Open access under CC BY-NC-ND license.

Peer review under responsibility of Faculty of Agriculture, Ain Shams University.
http://dx.doi.org/10.1016/j.aoas.2012.03.002

Production and hosting by Elsevier

variations as taxonomic characters to define identify *Kalanchoe* species in this study.

Materials and methods

Fifteen species of *Kalanchoe* were freshly collected from the garden of the agricultural Botany department, Faculty of Agriculture, Al-Azhar University. The following species were studied.

1 – *Kalanchoe beauverdii* Hamet	2 – *K. beharensis* Drake et Castillo
3 – *K. blossfeldiana* V. Poellnitz	4 – *K. caniflora* Adans
5 – *K. daigremontiana* Hamet & Perrier	6 – *K. fedtschenkoi* Hamet & Perrier
7 – *K longiflora* Adans	8 – *K. marmorata* Baker
9 – *K. pinnata* Pers.	10 – *K. pumila* Baker
11 – *K. roseleaf* Adans	12 – *K. serrata* L.
13 – *K. thyrsiflora* Raym & Hamet	14 – *K. tomentosa* Baker
15 – *K. tubiflora* (Harvy)Hamet	

The identification of the collected plants was achieved by comparing their morphological characters with the characters of the previously identified plants as published by Bailey (1951) and Longwood (2010).

Material treatment

Fresh stems and leaves samples were taken from young parts then 1 cm long from the middle part of the technical length of the stem and 1 cm^2 from leaf was taken. Samples were dehydrated in a series of solutions of ascending concentrations of ethyl alcohol varying from 50% to 100% ethyl alcohol. The samples then embedded in paraffin wax [m.p. 58–61 °C] using xylol as a solvent. By using rotary microtome, sections were cut at the thickness of 15 μm and then mounted on slides with the aid of egg albumin as an adhesive. Wax dissolved in xylol and the slides were passed through descending series of ethyl alcohol solutions varying from 100% to 50% ethyl alcohol concentrations in descending order. The sections on the slides 15 μm thick were stained with safranin and light green, and then the colored sections were kept as permanent preparations on the slides with canada balsam as mounting medium (Pandey, 1996) and examined by light microscope Carl Zeiss then photoed by eye piece digital camera (Hirocam 5).

Results and discussion

Stem anatomy

Data in Table 1 and Figs. 1–18 indicate that, cuticle layer was relatively thick in eight taxa as in *K. tubiflora* (Fig. 1) and thin in other taxa, hypodermis recorded in most of examined taxa as in *K. daigremontiana* (Fig. 2), the cork tissue noticed only in two taxa *K. blossfeldiana* and *K. beharensis* (Fig. 3). Three types of trichomes observed on the epidermal surface of three species, glandular hairs in *K. beharensis* (Fig. 3), multicellular branched hairs in *K. tomentosa* (Fig. 4) and papilla hairs in *K. caniflora* (Fig. 5). Pigmented epidermal cells were noticed only in *K. longiflora* (Fig. 6). Cortex was broad in some taxa as in *K. pumila* (Fig. 7) or narrow in the rest as in *K. tubiflora* (Fig. 8), cortex of most examined taxa with strongly developed collenchymatous cells as in *K. tubiflora* (Fig. 1). Storage cells (mucilaginous cells) were observed in all studied taxa except *K. beauverdii* but they were diffracted in density from highly density as in *K. beharensis* (Fig. 3) or low density as in *K. longiflora* (Fig. 6). Cortical vascular bundles were presented in three taxa such as in *K. roseleaf* (Fig. 9), secretory canals were noticed in some species as in *K. beharensis* (Fig. 10), druses crystals were recorded in some samples as in the last figure. Amorphous inclusion was presented only in K. *blossfeldiana* (Fig. 11). There were three types of pericycles noticed, pericycle with collenchymatous cells in some taxa as in *K. caniflora* (Fig. 12), pericycle with sclerenchymatous cells which was represented only in *K. tomentosa* (Fig. 13) and pericycle with cellulose fiber which was noticed in two taxa only as in *K. blossfeldiana* (Fig. 14). Storage cells were observed in the pericycle of some taxa as in *K. fedtschenkoi* (Fig. 15). Xylem vessels were in clusters in all examined taxa except *K. thyrsiflora* (Fig. 16) which was found in ring, collenchymatous bundle sheath recorded only in *K. caniflora* (Fig. 12). Medullary ray was recorded in all the examined samples except *K. thyrsiflora* (Fig. 16), in other taxa medullary ray recognized by the presence of storage cells in six taxa as in *K. fedtschenkoi* (Fig. 15) and druses crystals noticed only in *K. beharensis* (Fig. 17). Pith also described in this study, it was opposite of cortex, when the cortex was broad pith was narrow, when the cortex was narrow pith was broad (see Figs. 7 and 8).Pith characteristic by the presence of collenchymatous cells in many samples as in *K. daigremontiana* (Fig. 18), storage cells as in *K. fedtschenkoi* (Fig. 15) and druses crystals as in *K. caniflora* (Fig. 12).

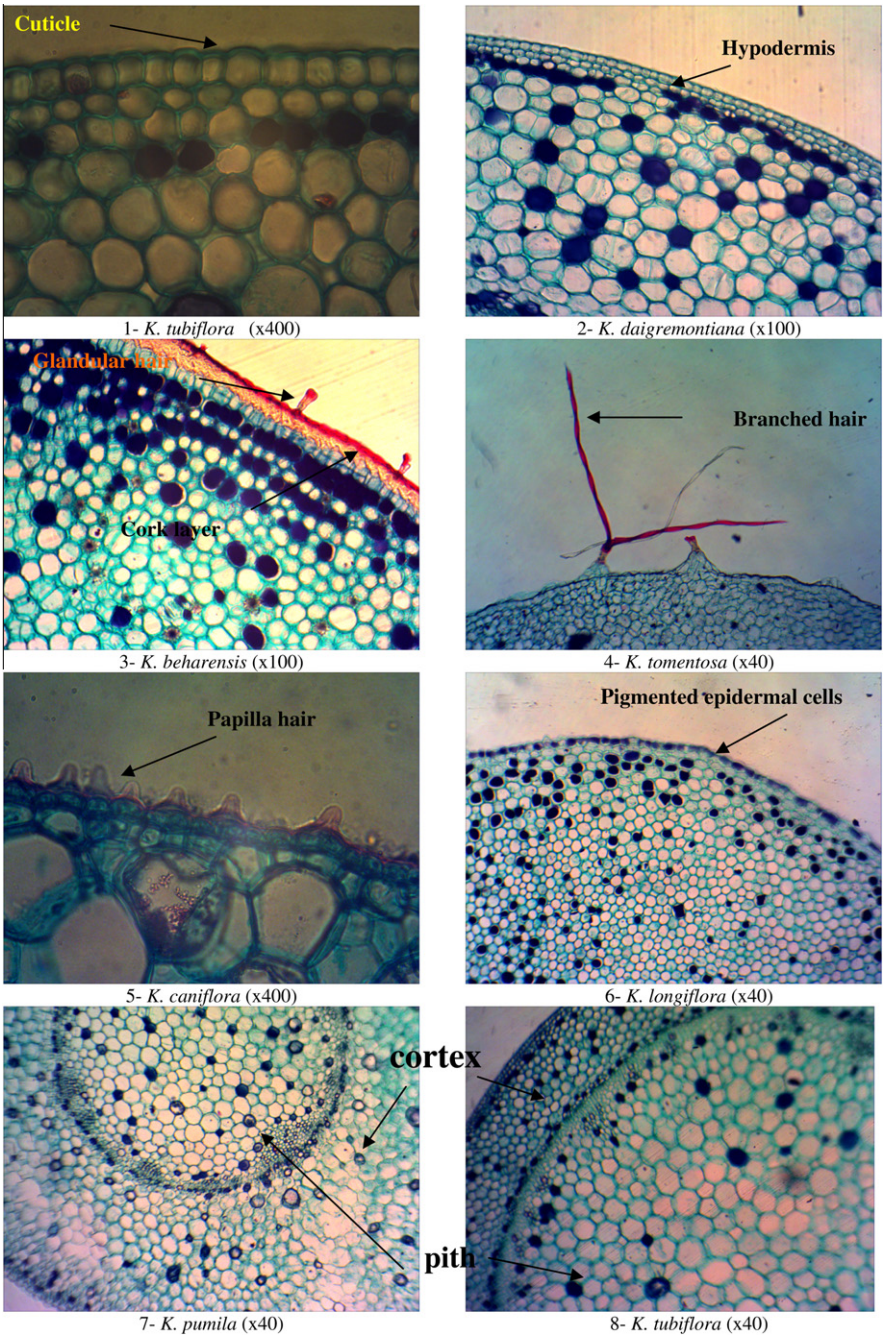
These results were in agreement with (Metcalfe and Chalk, 1950) who recorded that, the cortex of stem in Crassulaceae is usually fleshy and strongly developed and consists of succulent parenchyma or contain weakly developed collenchymas, the cork in *Bryophyllum* (*Kalanchoe*) and *Sedum* is developed in the epidermis or in other cases in the sub- epidermal layer or in a deeper layer of the primary cortex becomes the phellogen. Also in agreement with (Ardelean et al., 2009) who recorded that, the external wall of *Sedum telephium* L. (Crassulaceae) is covered by a thin cuticle, the fleshy aspect of the stem comes from a well developed parenchymatic tissue which occurs in the cortex and pith, also with Watson and Dallwitz (1992) who recorded the presence of cork cambium in some members of Crassulaceae deep seated (rarely) or superficial and cortical vascular bundles (commonly).

Leaf anatomy

Table 1 and Fig. 19–28 showed that, cuticle layer may be thick as in *K. thyrsiflora* (Fig. 19) or thin in most examined taxa such as in *K. beharensis* (Fig. 20), large epidermal cells noticed only in *K. beauverdii* (Fig. 21), hypodermis recorded only in two samples, in *K. beharensis* and *K. tubiflora* (Fig. 22). Multicellular branched hairs observed only on the epidermal surface of two samples, *K. tomentosa* and *K. beharensis* (Fig. 23). Although the mesophyll in genus *Kalanchoe* is homogenous and consists of large parenchymatous cells, this study showed that two taxa have heterogeneous mesophyll, *K. tomentosa* and *K. beauverdii* (Fig. 21) also, mesophyll characterized by the presence of storage cells in most examined taxa as in

Table 1 Data matrix of the observed characters in the different species. The following data-matrix based on 42 characters of 15 *Kalanchoe* species, the species arranged (1–15) as mentioned in material and method.

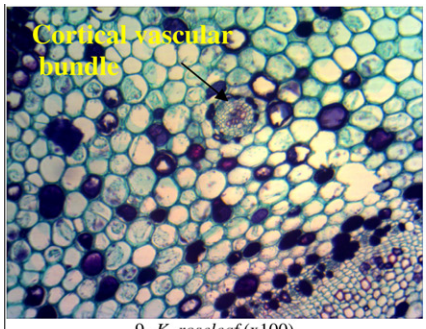
Leaf characters	Vascular bundle	42. Storage cell in xylem parenchyma present + absent -	'	'	+	'	+	+	+	'	'	+	+	'	+	'	'
		41. Storage cells sheath present +/absent	'	'	'	'	+	'	+	'	+	+	+	'	+	+	'
		40. Collenchy matous sheath present + / absent	+	+	+	'	+	+	'	'	+	+	+	'	+	+	+
		39. Xylem vessels in crescent shape + / in ring shape	+	+	+	+	+	+	'	+	+	+	+	+	+	+	+
	Midrib region	38. Storage cells present + / absent -	'	+	+	'	'	'	+	+	'	+	'	'	+	+	'
		37. thick more than blade + (not so -	+	'	'	'	'	'	'	'	'	+	'	+	+	'	'
	Mesophyll	36. druses crystals present + / absent -	+	+	'	'	'	'	'	'	'	'	'	'	'	+	'
		35. canals present + / absent -	'	'	'	'	'	'	'	+	'	'	'	'	'	'	'
		34. Storage cells present + / absent -	'	'	+	'	'	+	+	+	+	+	+	'	+	+	+
		33. homogenous + / heterogenous -	'	+	+	+	+	+	+	+	+	+	+	+	+	'	+
	Epidermis	32. Multicular hairs present + / absent -	'	+	'	'	'	'	'	'	'	'	'	'	'	+	'
		31. Hypodermis present + / absent -	'	+	'	'	'	'	'	'	'	'	'	'	'	'	+
		30. Large epidermal cell present + / absent -	+	'	'	'	'	'	'	'	'	'	'	'	'	'	'
		29. Cuticl thick + / thin -	'	'	+	'	+	+	'	'	+	'	'	'	'	'	+
Stem characters	Pith	28. Druses crystals present + / absent -	+	+	'	+	'	'	'	'	'	'	'	'	'	+	'
		27. Storage cells present + / absent -	'	+	+	'	'	+	+	+	+	+	+	+	'	+	+
		26. Collenchymatous cells present + / absent -	+	'	+	+	+	'	+	+	+	+	+	+	+	+	'
		25. broad + / narrow -	+	'	+	'	+	+	'	'	'	'	+	+	+	+	+
	Medulary ray	24. Druses crystals present + / absent -	'	+	'	'	'	'	'	'	'	'	'	'	'	'	'
		23. Storage cells present + / absent -	'	+	+	'	'	+	'	'	'	'	+	+	'	'	+
		22. present + / absent -	+	+	+	+	+	+	+	+	+	+	+	+	'	+	+
	Vascular bundle	21.bundle sheath present + / absent	'	'	'	+	'	'	'	'	'	'	'	'	'	'	'
		20. Vessels in clusters + / not so-	+	+	+	+	+	+	+	+	+	+	+	+	'	+	+
	Pericycl	19. Storage cells present + / absent -	'	'	+	'	+	'	+	'	+	'	+	'	'	'	'
		18. Cellulose fiber present + / absent -	'	'	+	'	'	'	'	'	'	'	'	+	'	'	'
		17. Sclerehchymatous cells present + / absent -	'	'	'	'	'	'	'	'	'	'	'	'	'	+	'
		16. Collenchymatous cells present + / absent -	+	+	+	'	+	'	'	+	+	'	+	'	'	'	'
	Cortex	15. Amorphous inclusion present + / absent -	'	'	+	'	'	'	'	'	'	'	'	'	'	'	'
		14.Druses crystals present + / absent -	+	+	'	'	'	'	'	'	'	+	'	'	'	+	'
		13. Canal present + / absent -	'	+	'	'	'	'	'	'	'	+	'	'	'	+	'
		12. Cortical bundles present + / absent -	'	+	'	'	'	'	'	'	+	'	+	'	'	'	'
		11. If present, highly density + / low density -	'	'	+	'	'	+	'	'	'	'	+	'	'	'	'
		10. Storage cells present + / absent -	'	+	+	+	+	+	+	+	+	+	+	+	+	+	+
		9. Collenchymatous cells present + / absent -	+	+	+	+	+	+	+	+	'	'	+	+	+	+	+
		8. Broad + / narrow -	'	+	'	+	'	'	+	+	+	+	'	'	'	'	'
	Epidermis	7. Pigmented cell present + / absent -	'	'	'	'	'	'	+	'	'	'	'	'	'	'	'
		6. Papilla present + / absent -	'	'	'	+	'	'	'	'	'	'	'	'	'	'	'
		5. Multicellular hairs present + / absent -	'	'	'	'	'	'	'	'	'	'	'	'	+	'	
		4. Glandular hairs present + / absent -	'	+	'	'	'	'	'	'	'	'	'	'	'	'	'
		3. Cork present + / absent -	'	+	+	'	'	'	'	'	'	'	'	'	'	'	'
		2. hypodermis present + / absent -	'	+	+	'	+	+	'	+	'	'	+	+	+	'	+
		1. Cuticle layer thick + /thin	+	+	'	'	+	+	'	+	'	'	'	'	+	+	+
		Characters spp.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15



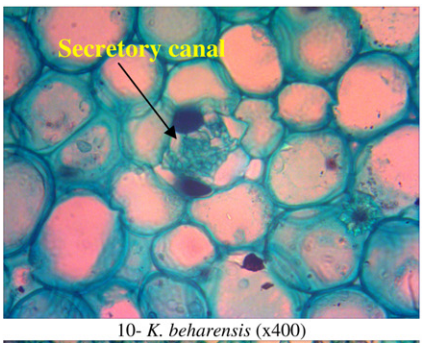
Figs. 1–18 Cross sections in stems of *Kalanchoe* spp.

K. tubiflora (Fig. 22), secretory canals founded in the mesophyll of *K. marmorata* (Fig. 24) and druses crystals noticed in few taxa as in *K. beharensis* (Fig. 25). In most examined samples the cross section was flat but it was circulate in *K. tubiflora*. In most studied species the midrib region is not clearly recognized in leaf blade but in four taxa only the midrib region was more thickness about blade as in *K. pumila* (Fig. 26), storage cells noticed also in the midrib region of some taxa (see Table 1). Xylem vessels in vascular bundles arranged in two shapes, crescent shape in all examined taxa as shown in *K. tomentosa* (Fig. 27) and in ring shape as in *K. longiflora* (Fig. 28). As well knowing kranz unite (clorenchymatous sheath) absent in CAM plants but the two types of sheathe recorded, collenchymatous sheath in most taxa as in *K. pumila* (Fig. 26) and storage cells sheath such as *K. longiflora* (Fig. 28) and storage cells noticed in xylem parenchyma in many species.

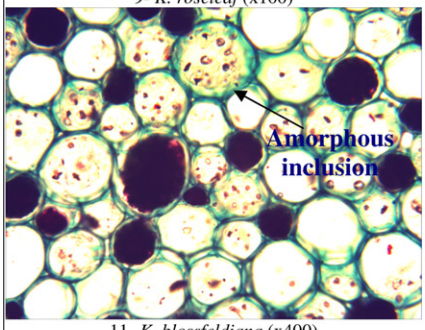
The results of this work are compatible with Smith et al. (1996), Winter and Smith (1996) who recorded that, general features of CAM leaf anatomy characterized by undifferentiated mesophyll cells. Also with Borland et al. (2000) who founded that, leaves of CAM plants with water storage tissues. (Balsamo and Uribe, 1988) described anatomically leaves of *K. dagremontiana* which were with thickened cuticle, presence of bundle sheath and mesophyll was not differentiated into



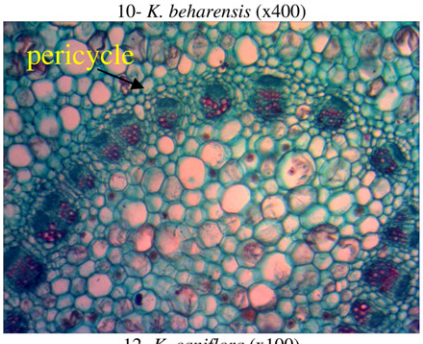
9- *K. roseleaf* (x100)



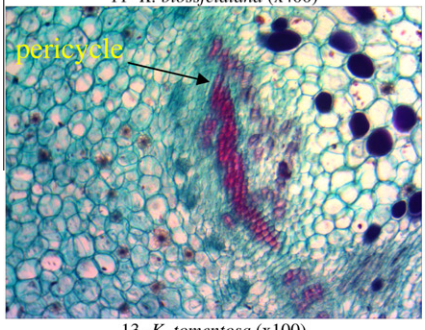
10- *K. beharensis* (x400)



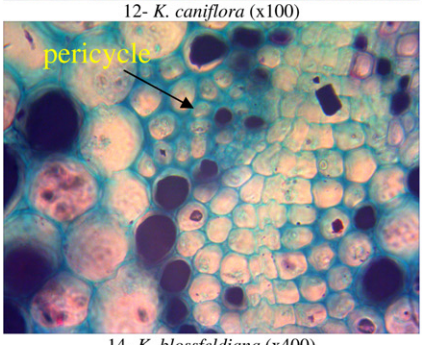
11- *K. blossfeldiana* (x400)



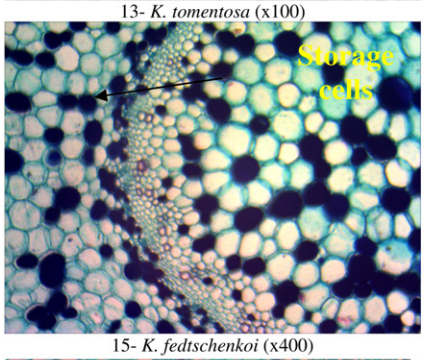
12- *K. caniflora* (x100)



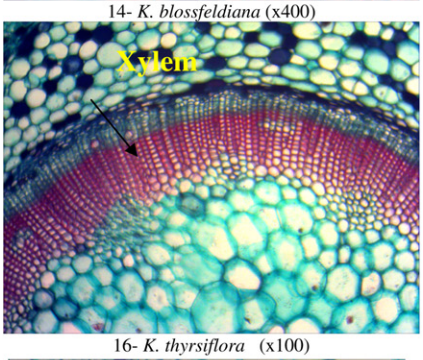
13- *K. tomentosa* (x100)



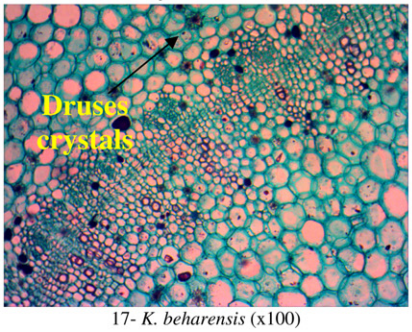
14- *K. blossfeldiana* (x400)



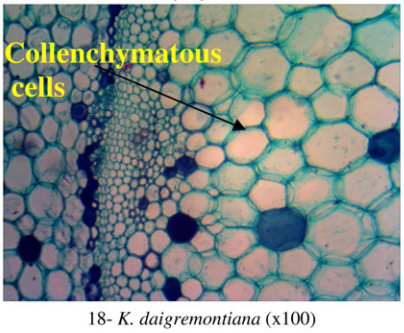
15- *K. fedtschenkoi* (x400)



16- *K. thyrsiflora* (x100)

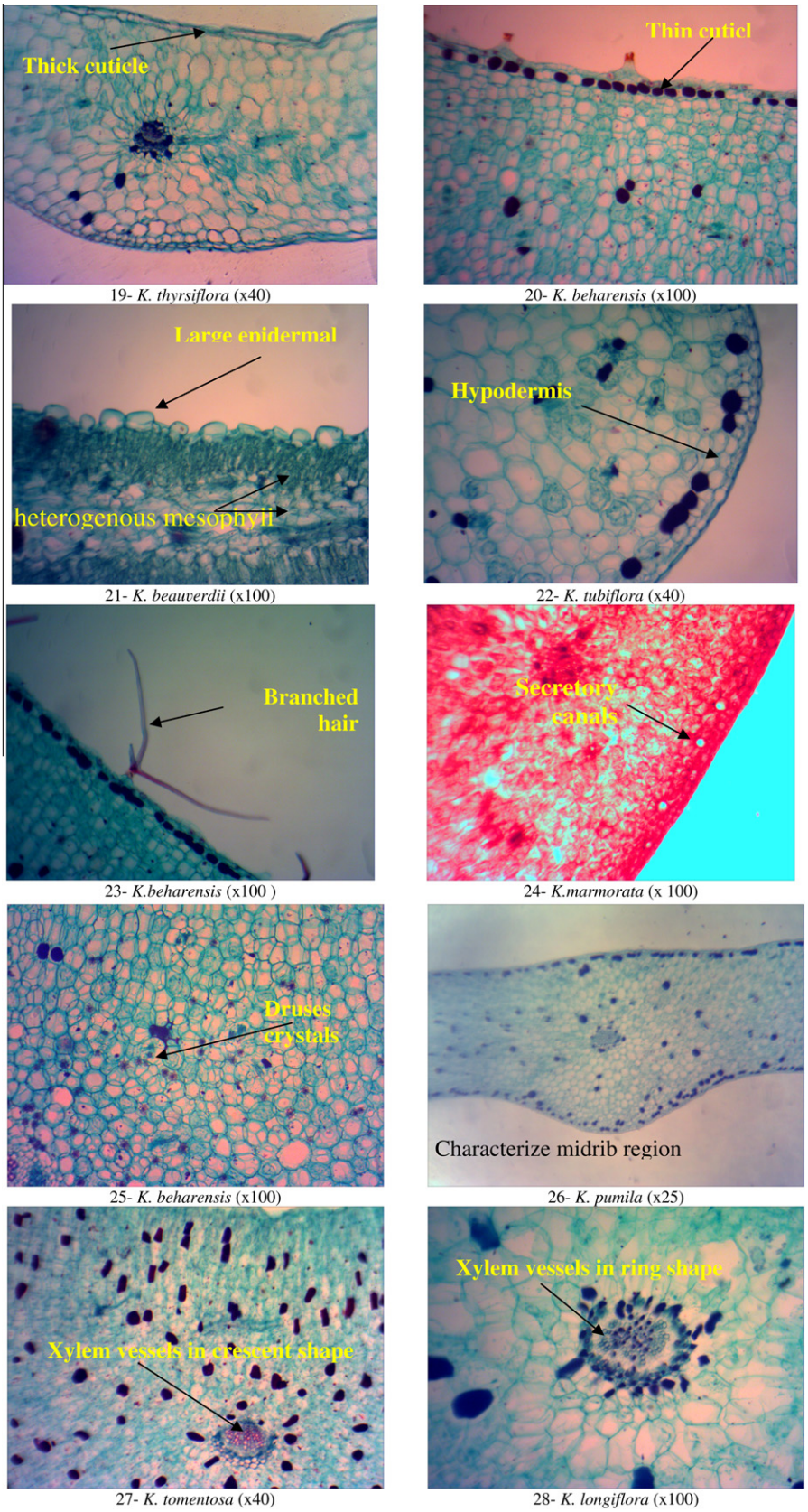


17- *K. beharensis* (x100)



18- *K. daigremontiana* (x100)

Figs. 1–18 (*continued*)



Figs. 19–28 Cross sections in leaves of *Kalanchoe* spp.

palisade and spongy parenchyma which in agreement with the present results. The presence of trichomes on the epidermis of *Seldom* (Crassulaceae) recorded by Hamet (1907, 1908), Solereder (1908), Rauh (1995) and Descoings (2003). Elzbieta and Mykaylo (2005) recorded non-glandular branched hairs on the epidermal surface of *K. beharensis* and *K. tomentosa* which similar with the same data in this work. Mykhaylo and Elzbieta (2008) investigated the anatomical structure of leaves in *K. pumila* which agreement with some present results but in contrary with the other, they agreement with the presence of storage cells in bundle sheath and xylem parenchyma and chlorenchymatic tissue was not differentiated into palisade and spongy tissue, they also added that, *K. pumila* with thick cuticle, vascular bundles were collateral and closed but in the present work in the same species the cuticle was thick and vascular bundle was open. This work gives the first report of the occurrence of papilla on the stem epidermis, collenchymatous bundles sheath in stem and mesophyll differentiated into palisade and spongy tissue in leaf.

Based on the observed anatomical features, indented key (Subrahmanyam, 1995) has been constructed to allow distinguishing the 15 species of *Kalanchoe*.

A. epidermal cells of stem with pigmented cells	*K. longiflora*
B. epidermal cells of stem without pigmented cells.	
* Hairs on stem surface present.	
1. Glandular hairs present	*K. beharensis*
2. Non-glandular hairs present	*K. tomentosa*
3. Papilla present	*K. caniflora*
** Hairs on stem surface absent	
a. Druses crystals in cortex present	
1 Canals absent	*K. beauverdii*
2 Canals present	*K. pumila*
b. Amorphous inclusion	*K. blossfeldiana*
C. Xylem vessels in clusters and medullary ray present storage cells, contain druses crystals	
a. Pericycle with storage cells	*K. roseleaf*
b. Pericycle without storage cells	*K. serrata*
D. Xylem vessels in ring, medullary ray absent	*K. thyrsiflora*
a. Cross section of leaf is circle	*K. tubiflora*
b. Cross section of leaf is flat	
* Large epidermal cells present	*K. beauverdii*
** Large epidermal cells absent	
e. Canals in mesophyll present	*K. marmorata*
ee. Canals in mesophyll absent	
+ Storage cells in mesophyll and bundle sheath Absent	*K. fedtschenkoi*
+ + Storage cells in mesophyll and bundle sheath present	
1. Storage cells present in xylem parenchyma but it is absent in pith and mesophyll	*K. diagremontiana*
2. Storage cells absent in xylem parenchyma and present in pith and mesophyll	*K. pinnata*

References

Ardelean M., Irina S. and Dorina C. 2009. Sedum telephium L. ssp. maximum (L.) Krock- Histo- anatomical aspects on the vegetative organs. Analele Stiinttifice Ale Universtatii''A1.I. Cuza''Iasi Tomul Lv, fase. 2, S.II a Biologie Vegetala. pp. 75–80.

Bailey, L.H., 1951. Manual of Cultivated Plants. The Macmillan pub. Co., London, pp. 454–469.

Balsamo, R.A., Ernest, U.G., 1988. Leaf anatomy and ultrastructure of the Crassulacean-acid-metabolism plant *Kalanchoe daigremontiana*. Planta 173, 183–189.

Borland, A.M., Maxwell, K., Griffiths, H., 2000. Ecophysiology of plants with Crassulacean acid metabolism. In: Leegood, R.C., Sharkey, T.D., von Caemmerer, S. (Eds.), Photosynthesis: Physiology and Metabolism. Kluwer Academic Publishers, The Netherlands, pp. 560–583.

Descoings, B., 2003. *Kalanchoe*. In: Eggli, U. (Ed.), Illustrated Handbook of Succulent Plants, Crassulaceae, Sukkulenten-Sammlung Zurich Mythenquai 88 8002 Zurich, Switzerland, pp. 143–181.

Elzbieta, W.C., Mykaylo, C., 2005. Structure of trichomes from the surface of leaves of some species of *Kalanchoe* Adans. Acta Biological Cracoviensia Series Botanica 47 (2), 15–22.

Hamet, R., 1907. Monographie du genera *Kalanchoe*. Bulletin de l Herbier Boissier 7, 869–900.

Hamet, R., 1908. Monographie du genera *Kalanchoe*. Bulletin de l Herbier Boissier 8, 17–48.

Kluge, M., Ting, I.P., 1978. Crassulacean Acid Metabolism. In: Analysis of an Ecological Adaptation, vol. 30. Springer-Verlag, Berlin-Heidelberg, pp. 70–74.

Longwood, L., 2010. How to Identify Kalanchoe Plants <www.ehow.com>.

Luttge, U., Ball, E., 1977. Water Relations parameters of the CAM.plants *Kalanchoe daigremontiana* in relation to diurnal malate oscillation. Oecologia 31, 85–94.

Metcalfe, C.R., Chalk, L., 1950. Anatomy of the Dicotyledons, vol. 2. Oxford at the Clarendon Press, UK, pp. 320–324.

Mykhaylo, C., Elzbieta, W., 2008. Structure of *Kalanchoe pumila* Bak. leaves. Acta Agrobotanica 61 (2), 11–24.

Pandey, B.P., 1996. Modern Practical Botany, vol. 2. S. Chand & Co., Ltd., Ram Nagar 110 055, New Delhi, India, pp. 1–11.

Rauh, W., 1995. Succulent Xerophytic Plants of Madagascar, vols. 1 and 2. Strawberry Press, Mill Valley, Calif., USA.

Smith, J.A.C., Ingram, J., Tsiantis, M., Barkla, B.J., Bartholomen, D.M., Bettey, M., Pantoja, O., Pennington, A.J., 1996. Transport across the vacuolar membrane in CAM plants. In: Winter, K., Smith, J.A.C. (Eds.), Crassulacean Acid Metabolism: Biochemistry, Ecophysiology and Evolution. pp. 53–71.

Solereder, H., 1908. Systematic Anatomy of the Diccotyledons. Clarendon Press, Oxford, UK, pp. 911–912.

Subrahmanyam, N.S., 1995. Modern Plant Taxonomy. Sangam Books Ltd., 110007 Delhi, India, p. 187.

Ting, I.P., 1982. Plant Physiology. Addison–Wesley Pub. Co., New York, USA, p. 642.

Warmbrodt, R.D., 1984. Structure of the leaf of *Pyrossia longiflora*, a fern exhibiting Crassulacean acid metabolism. American Journal of Botany 71, 330–347.

Watson, L., Dallwitz, M.J., 1992. The families of flowering plants, descriptions, illustrations, identification and information retrieval. <www.delta-intkey.com>.

Winter, K., 1985. Crassulacean Acid Metabolism. In: Photosynthetic Mechanisms and the Environment. Elsevier Pub. Co., Amsterdam, pp. 329–387.

Winter, K., Smith, J.A.C., 1996. An Introduction to Crassulacean Acid Metabolism: Biochemical Principles and Ecological Diversity. In: Winter, K., Smith, J.A.C. (Eds.). Springer, Berlin, pp. 1–10.