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COMPARATIVE MORPHO-ANATOMICAL ANALYSIS OF *KALANCHOE* ADANS. SPECIES FROM BANGLADESH

MD ASHIKUR RAHMAN LASKAR, MD TAHIDUL ISLAM, MD MAHADIY HASAN, SUBROTO KUMAR DAS AND MD UZZAL HOSSAIN*

Department of Botany, University of Barishal, Barishal-8254, Bangladesh

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Abstract

Morpho-anatomy of five *Kalanchoe* species including firstly reported *Kalanchoe gastonis-bonnieri* and *Kalanchoe delagoensis* species found in Bangladesh was analyzed to elucidate the previous taxonomic dispute by comparative study. Stomata observed in leaves of all of the experimented species were amphistomatic in nature. Presence of unequal subsidiary cells surrounding the guard cells was the cellular morphology of all the stomata. The abaxial surface of leaves contained more stomata than the adaxial surface in each species. *K. gastonis- bonnieri* and *K. delagoensis* were found to have the highest (53.4 ± 1.88 stomata/mm^2) and the lowest (14.81 ± 0.53 stomata/mm^2) stomatal density respectively. Many dissimilarities were observed during the anatomical study of leaves and stems. The leaf epidermis of the examined species was lack of multicellular hair and mesophyll tissue was not differentiated into palisade and spongy parenchyma. Crescent shaped vascular bundles were surrounded in the midrib regions of leaves in all species examined except *Kalanchoe blossfediana*. Existence of collenchymatous sheath around the midribs vascular bundles and absence of storage cells were characteristics of leaves midribs. Thick cuticular layer on the epidermis except *Kalanchoe pinnata*, a well-developed broad cortex except *K. delagoensis* and well defined pith were found to be characteristic of stem along with lack of cortical resin canal and storage cells in medullary rays.

Introduction

The genus *Kalanchoe* Adans. one of the important genera in Crassaulaceae comprises of about 125 species (Wojtasiak *et al.* 2019) and native to South Africa but has a worldwide distribution in tropical climate as exotic or invasive species (Richwagen *et al.* 2019). Diverse array and strange beauty of the plants from this genus attracted public interest and consequently makes available as ornamental household, succulent garden plants (Czepas *et al.* 2016).

Hooker (1886) documented eight genera including 40 species from the Indian subcontinent of which two species were explored from Bangladesh territory from Crassulaceae (Rahman and Rashid 2012). Ahmed *et al.* (2008) reported five species namely *Kalanchoe blossfeldiana, Kalanchoe daigremonitnum, Kalanchoe heterophylla, Kalanchoe laciniata* and *Kalanchoe pinnata* from Bangladesh already. Morpho-taxonomical study on *Kalanchoe* makes this genus as an intricate genus, which is not clearly delimitated yet (Perez *et al.* 2020). Throughout the literature published concerning both in its nomenclature and systematics two contrary tendencies have been reported. The authors oppose whether or not the classification is primarily based totally on a single genus called *Kalanchoe* or on three different sections: *Kalanchoe*, *Bryophyllum* Kahl., and *Kitchingia* because of diverse evolutionary contentions, morphological features and molecular analyses (Perez *et al.* 2020). The three sections view of *Kalanchoe* instead of well prevalent exhibit variations in flower morphology and geographical distribution. The species of the section *Kalanchoe* generally tend to have erect flowers and connate stamens at center of the tubular corolla, while the contributors of

*Author for correspondence: <muhossain@bu.ac.bd>.

the section *Bryophyllum* usually bears bulbils along their leaf margins, pendant flowers, and an inflated tube of fused sepals with basal position of stamens at the corolla tube. On the other hand, species of the section *Kitchingia* share the constant flower morphology with the section *Bryophyllum* and therefore the alike stamen position with the section *Kalanchoe*, with distinct spreading carpels of floral structure. Study of the comparative morphology may be enough in setting those species into proper taxonomic ranks because of the presence of similarities and variations in both within and across sections. But the taxonomic status of these three sections were not clear when the genus *Kalanchoe* was first published (Baldwin 1938). This aspect raises a few issues in classification of the genus from time to time and consequently additional supportive features are needed.

In determining species delimitation and resolving taxonomic dispute micromorphological traits may be substantial. The taxonomic importance of vegetative anatomy along with structure of epidermal cells in taxa demarcation and intergeneric or interspecific connection has been widely documented (Karaismailoglu 2019). Differentiation between plant groups is aided by foliar anatomy as the leaf is the most morphologically diverse organ in angiosperms and provides number of anatomical traits that can be used as valuable taxonomic tools. In addition, numerous features in stem and leaf anatomy have already been demonstrated to be of relevance in classification and have been frequently used in systematics research (Sultana *et al*. 2019). As external characteristics especially vegetative ones may vary due to heterogeneous environmental condition. So microscopic and internal anatomical features may be used as basic tool for authentic identification of each species from this genus. Despites this is the best known genus of the family Crassualaceae for botanical and medicinal importance (Keeley and Philip 2003), no anatomical study on this five *Kalanchoe* species has been conducted in Bangladesh so far. Therefore, the present study was aimed to explore the comparative internal anatomy of leaf and stem of these *Kalanchoe* species for delimitation of the species relationship.

Materials and Methods

Plant specimens named *K. blossfeldiana* V. Poelln., *K. delagoensis* Eckl. And Zeyh., *K. gastonis-bonnieri* Raym.-Hamet and H. Perrier, *K. laciniata* (L.) DC. and *K. pinnata* (Lamk.) Pers. (Fig. 1 A-E) were collected from different districts of Bangladesh and critically studied along with identification with the help of a comprehensive literature review on genus. Updated nomenclature of the species were confirmed with consulting Encyclopedia of Flora and Fauna of Bangladesh (Ahmed *et al.* 2008), illustrated handbook of succulent plants (Eggli 2003), the nomenclatural databases of The Plant List (2013) and TROPICOS (2017).

Micro-morphology of leaves were carried out by handmade paradermal sections from both sides of the fresh leaves. Peeled off leaf epidermis was placed on a glass slide with 33% glycerin and covered with a glass slip. The prepared materials were examined under Olympus CH30 light microscope. The density of epidermal cells and stomata per mm^2 was observed. Based on the number of epidermal cells and stomata per leaf unit area, the stomatal index was calculated. It expresses the percentage of stomata to the number of epidermal cells within the analyzed area, calculated by the following (Chernetskyy *et al*. 2018) formula:

$$\text{Stomatal index (SI)} = \frac{S}{S+E} \times 100$$

Where, S is the number of the stomata/mm^2 and E is the number of the epidermal cells/mm^2.

Anatomy of leaf and stem was performed by transverse sections (TS) using a sharp blade. The fine sections were kept in a watch glass with diluted safranin. Then the colored sections were

placed on the slides with glycerin solution for mounting and examined by Olympus CH30 light microscope. The photographs were taken by means of a Euromex (CMEX-18Pro) eyepiece digital camera attached to the microscope.

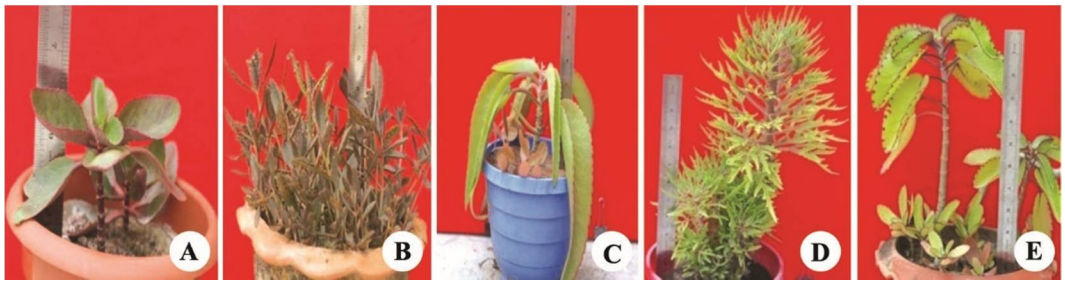

Fig. 1. Five specimens of *Kalanchoe*. A: *K. blossfeldiana*; B: *K. delagoensis*; C: *K. gastonis-bonnieri*; D: *K. laciniata* and E: *K. pinnata*.

Results and Discussion

Leaves of all studied species were amphistomatic with anisocytic stomata. Moreover, all of these species had fully functional stomata along with some rudimentary stomata. Chernetskyy (2012) found amphistomatic leaves with anisocytic stomata in all the studied 35 *Kalanchoe* species. The coexistence of rudimentary stomata with functional ones was also reported in *K. pinnata* and *K. crenata* (Moreira *et al*. 2012), which is related to the ability of succulent leaves to undergo cell division long after the leaves are photosynthetically active (Ting and Gibbs 1982). In each species, the abaxial surface had more stomata than the adaxial surface. Epidermal cells on the adaxial surface were always larger in size than that present on the abaxial surface, as evidenced by their number per unit leaf area. Usually abaxial surfaces had a higher stomatal index (SI) than the adaxial surfaces except *K. blossfeldiana*. Stomatal density in the studied five *Kalanchoe* species were found to 14.81 ± 0.53 and 53.4 ± 1.88 stomata/mm^2 in *K. delagoensis* and *K. gastonis-bonnieri*, respectively (Table 1). The presence of higher number of stomata in the abaxial surface of leaf was confirmed by Chernetskyy *et al*. (2018) and Moreira *et al.* (2012) in *K. daigremontiana* and in *K. pinnata* and *K. crenata* respectively. Ting and Gibbs (1982) estimated that CAM plants commonly had stomatal densities varying from 10 to 65mm^{-2}, which is supportive of the present findings.

Table 1. Comparative stomatal density of *Kalanchoe* species.

Species	Stomatal density, S (Stomata/mm^2)		Density of epidermal cells, E (Cells/mm^2)	
	Adaxial	Abaxial	Adaxial	Abaxial
K. blossfeldiana	18.21 ± 1.11	25 ± 1.41	80.56 ± 8.35	125 ± 18.98
K. delagoensis	14.81 ± 0.53	19.75 ± 0.82	93.21 ± 1.72	115.12 ± 2.94
K. gastonis-bonnieri	31.48 ± 7.43	53.4 ± 1.88	108.64 ± 9.7	191.67 ± 5.35
K. laciniata	19.75 ± 3.13	28.7 ± 0.53	100.62 ± 3.56	116.36 ± 6.15
K. pinnata	32.41 ± 0.93	49.38 ± 1.35	127.16 ± 3.44	140.43 ± 4.01

All values are expressed as mean ± standard error.

Among the five studied species, *K. delagoensis* and *K. laciniata* had thick cuticle layers on the leaf epidermis, while the remaining species possessed thin cuticular layers. Leaf hypodermis

was found in *K. blossfeldiana*, *K. delagoensis* and *K. pinnata*. Large epidermal cells and multicellular hairs were absent in all the studied specimens alike stem epidermis (Fig. 2; Table 2). Mesophyll tissue was found to be uniform in all species which means, it could not be differentiated into palisade and spongy parenchyma. Thick midrib regions were found only in *K. pinnata*. Xylem vessel in all species were oriented in a crescent shape except *K. blossfeldiana*. Collenchymatous sheath around the leaf vascular bundle were found in every species observed. All parts of the leaf of the studied *Kalanchoe* species were devoid of storage cells (Fig. 2; Table 2).

Table 2. Data matrix of the observed anatomical characters of leaf and stem.

Region	Sub-region	Characteristics	Species				
			KB	KD	KG	KL	KP
Stem	Epidermis	Cuticle layer thick + / thin -	+	+	+	+	-
		Hypodermis present + / absent -	+	+	+	-	+
		Cork present + / absent -	-	-	-	-	-
		Multicellular hairs present + / absent -	-	-	-	-	-
		Pigmented cells present + / absent -	+	+	+	-	-
	Cortex	Broad + / narrow -	+	-	+	+	+
		Collenchymatous cells present + / absent -	-	-	+	+	-
		Storage cells present + / absent -	+	-	+	-	+
		Canal present + / absent -	-	-	-	-	-
	Pericycle	Collenchymatous cells present + / absent -	+	+	+	-	-
		Sclerenchymatous cells present + / absent -	-	+	-	+	+
		Storage cells present + / absent -	-	-	+	+	-
	Medullary cells	Present + / absent -	+	-	-	+	+
		Storage cells present + / absent -	-	-	-	-	-
	Pith	Broad + / narrow -	-	+	-	-	+
		Collenchymatous cells present + / absent -	-	-	-	+	-
		Storage cells present + / absent -	-	-	+	-	-
Leaf	Epidermis	Cuticle thick + / thin -	-	+	-	+	-
		Hypodermis present + / absent -	+	+	-	-	+
		Large epidermal cells present + / absent -	-	-	-	-	-
		Multicellular hairs present + / absent -	-	-	-	-	-
	Mesophyll	Storage cells present + / absent -	-	-	-	-	-
		Differentiated into palisade and spongy parenchyma + / not so -	-	-	-	-	-
	Midrib region	Thick more than blade + / not so -	-	-	-	-	+
		Storage cells present + / absent -	-	-	-	-	-
	Vascular bundle	Xylem vessels in crescent shape + / ring shape -	-	+	+	+	+
		Collenchymatous sheath present + / absent -	+	+	+	+	+
		Storage cells sheath present + / absent -	-	-	-	-	-

KB = *K. blossfeldiana*, KD = *K. delagoensis*, KG = *K. gastonis-bonnieri*, KL = *K. laciniata* and KP = *K. pinnata.*

Being succulent plants, their stems were mostly covered with thick cuticle except *K. pinnata*, which have a thin cuticular layer. Hypodermis was found in the stem of all species examined except *K. laciniata*. None of these investigated species were found to possessed cork tissue and

multicellular hairs on the epidermis. Pigmented epidermal cells were observed in *K. blossfeldiana, K. delagoensis* and *K. gastonis-bonnieri.* All *Kalanchoe* species studied had a broad cortex except

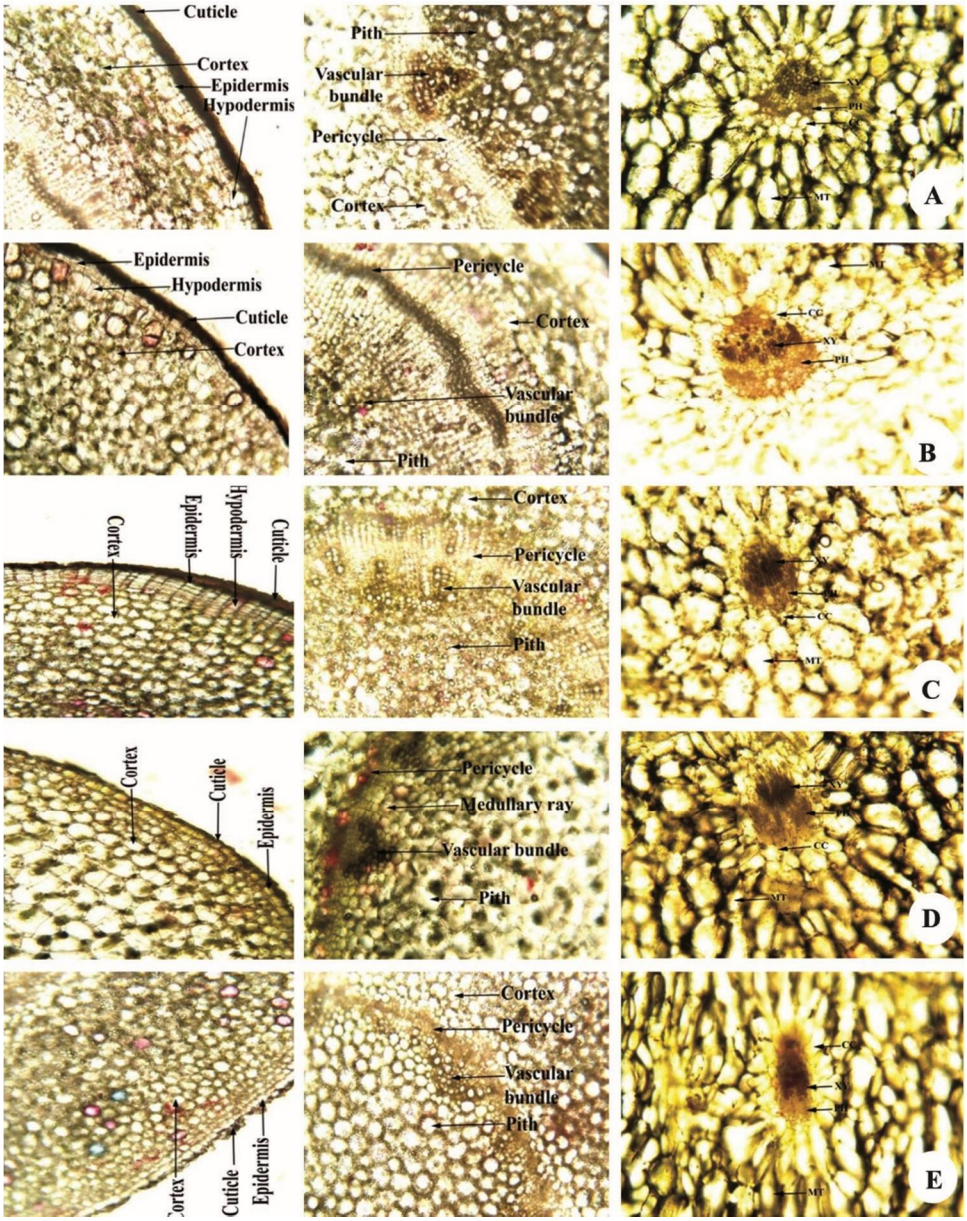

Fig. 2. TS of *Kalanchoe* stems and leaf midribs. A- *K.blossfeldiana*, B- *K. delagoensis*, C- *K. gastonis-bonnieri*; D- *K.laciniata*, E- *K.pinnata*

K. delagoensis (Fig. 2). In *K. gastonis-bonnieri* and *K. laciniata*, collenchymatous cells were observed in the cortical region. Storage cells within the cortical region were found in *K. blossfeldiana*, *K. gastonis-bonnieri* and *K. pinnata*. All the studied species were found to lack of a

resin canal in the cortical region of stem. Collenchymatous cells in the pericycle were found in all species except *K.laciniata* and *K. pinnata.* On the other hand, sclerenchymatous cells were observed in *K. delagoensis, K. laciniata* and *K. pinnata*. Presence of storage cells in the pericycle was found in *K. gastonis-bonnieri* and *K. laciniata* (Fig. 2; Table 2). Though medullary ray was a common feature of dicot plants but *K. delagoensis* and *K. gastonis-bonnieri* lack of it. Storage cells in medullary rays were not observed in any specimen. Central pith of *K. delagoensis* and *K. pinnata* were broader than others. Among all the species, only *K. laciniata* had collenchymatous cells in its pith. Storage cells in the pith region were observed in *K. gastonis-bonnieri* only (Fig. 2; Table 2).

The present results are supportive of Metcalfe and Chalk (1950), who recorded that the cortex of the stem in Crassulaceae is usually fleshy and strongly developed and consists of succulent parenchyma or contains weakly developed collenchymas tissues. Kluge and Ting (1978) also reported that mesophyll cells of CAM plants were not usually differentiated into palisade and spongy parenchyma. Most of the findings of Abdel-Raouf (2012) also supported the data obtained in the present work. However, he found cork tissue in stem epidermis and collenchymatous cells in the cortex of *K. blossfeldiana* which were absent in this study. Based on the observed anatomical features (Table 2), an indented key (Subrahmanyam 1995) was constructed to distinguish the five species of *Kalanchoe* following-

A. Stem epidermis with thin cuticle layer	*K. pinnata*
B. Stem epidermis with thick cuticle layer	
I. Narrow stem cortex	*K. delagoensis*
II. Broad stem cortex	
a. Medullary ray absent	*K. gastonis-bonnieri*
b. Medullary ray present	
I. Collenchymatous cells present in pith	*K. laciniata*
II. Collenchymatous cells absent in pith	*K. blossfeldiana*

The present morpho-anatomical profiling of five *Kalanchoe* species from Bangladesh is the first study that clearly shows significant differences. Sometimes comparative leaf anatomy, viz. cuticle, hypodermis, hairs, storage cells, mesophyll tissue and vascular bundle appeared as useful for diagnostic tools and in case of stem anatomy *viz.* cortical layers, resin canal, pericycle, medullary rays and pith are used as identification materials at the respective stage of development. So the present study will provide baseline data for morphological profiling along with the molecular characterization.

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