JJBS

Jordan Journal of Biological Sciences

Volume 10, Number 3,September 2017
ISSN 1995-6673
Pages 185-191

Comparative Anatomy of Stem, Petiole and Flower Stalks and its significance in the Taxonomy of Some Members of Cucurbits

Chimezie Ekeke*, Ikechukwu O. Agbagwa and Alozie C. Ogazie

Department of Plant Science and Biotechnology, Faculty of Science, University of Port Harcourt, P.M.B. 5323, Port Harcourt, Rivers State, Nigeria.

Received, January 26, 2017; Revised May 10, 2017; Accepted May 27, 2017

Abstract

We compared the anatomy of the flower stalk (male and female) anatomy, stem, petiole, tendrils and median vein shape of three representative members of Cucurbitaceae (*Citrullus lanatus*, *Cucumeropsis mannii* and *Citrullus colocynthis*). These materials (flower stalks of the male, female, midrib, petiole and the tendril) were fixed in FAA, wax embedded, sectioned, stained and photographed with Leitz Diaplan photomicroscope fitted with Leica WILD MPS 52 camera. The midrid and lamina anatomy of these species showed clear variations in the layers of mesophyll, number and arrangement of vascular bunbles which are of taxonomic significance and are reported for the first time. These anatomical features suggest taxonomic affinity amongst the species and enhance the delimitation of the species. It also strengthens the fact that the species maintained as separate species and confirm the fact that the anatomical characteristics of the stem, petiole, flower stalks, midrib and leaf lamina are an important line of evidence in classification of these species.

Keywords: Anatomy, Cucurbitaceae, Cucurbit, Flower Stalk, Midrib, Vascular Bundles.

1. Introduction

Cucurbitaceae is a group of flowering plants with 100 genera and 750 species (Yamaguchi, 1983). These plants are widely distributed in the tropical part of the world. They are herbs or rarely undershrubs with watery juice, often scabrid; stems scandent or prostrate; tendrils mostly present, spirally coiled. Flowers are monoecious or dioecious, very rarely hermaphrodite and actinomorphic. Male flower: calyx tubular, lobes imbricate or open; corolla polypetalous or gamopetalous, lobes imbricate or induplicate-valvate; stamens free or variously united, mostly 3, rarely 1–5, one anther always 1-celled, the others 2-celled, cells straight or often curved, flexuous or conduplicate, connective often produced. Female flower: calyx-tube adnate to the ovary and often produced beyond it; staminodes usually not present; ovary inferior or very rarely free; placentas often 3, parietal but often meeting in the middle; ovules numerous, rarely few, arranged towards the walls of the ovary; style simple or rarely 3 free styles; stigmas thick. Seeds various, often flattened, without endosperm (Hutchinson and Dalziel, 1952).

Leaves, roots and fruits of members of this family are used in Nigeria and other African countries for different purposes (food and medicine) depending on the ethnic group (Ajuru and Okoli, 2013; Aguoru and Okoli, 2008; Burkill, 1985; Okoli, 1984; Aguoru and Ogaba, 2010; Jansen van Rensburg *et al*., 2004). Among other plant species and cucurbits, anatomical characteristics of the petiole, leaf lamina, midrid, flower stalks and the number of vascular bundles in the different parts of the plants have been demonstrated to be valuable in delimitating the plants of the same genus or family (Ekeke and Agbagwa, 2016; Ekeke, *et. al.*, 2016; Ekeke and Mensah, 2015; Agogbua *et al.*, 2015; Ekeke *et al.*, 2015; Ajuru and Okoli, 2013; Aguoru and Okoli, 2012). Although a number of recent studies have been carried out on the phylogenetic relationship among cucurbits, the significance of fruit stalks, petiole, midrib and tendril anatomy in the taxonomy of these species from Nigeria is lacking. The present work, therefore, investigates the comparative anatomy of these plant parts to enhance the delimitation of these species.

2. Materials and Methods

2.1. Source of Material

Seeds of properly identified plants (*Citrullus colocynthis* (L.) Schard, *Cucumeropsis mannii* Naud and *Citrullus lanatus* (Thunb.) Matsum and Nakai were harvested in September 2015, processed and stored. In February 2016, the seeds were germinated in pots and the vegetative parts, used for the present study, were harvested at maturity (when the plants had started flowering and fruiting).

* Corresponding author. e-mail: ekeke.uche@uniport.edu.ng.

2.2. Anatomical Studies

The flower stalks of the male and female flowers, petioles (distal – base of the leaf, median – middle of the petiole and proximal – petiole portion close to the stem), median portion of the midrib and stems of plants were excised and fixed in FAA for 24hrs, dehydrated in 30%, 50% and 70% ethanol for 1hr each and preserved in absolute ethanol till when needed. Thereafter, the specimens were wax embedded, trimmed, and hand-sectioned. Thin sections selected, stained with 1% safranin or alcian blue, mounted on slide, observed under microscope and micro-photographed using Leica WILD MPS 52 microscope camera on Leitz Diaplan microscope (Ndukwu and Okoli, 1992; Ajuru and Okoli, 2012).

3. Results

The comparative anatomical characteristics of the stem, flower stalk (female and male), tendril, petiole and midrib of three species of cucurbits (*Citrullus lanatus*, *Cucumeropsis mannii* and *Citrullus colocynthis*) are summarized in Tables 1 and 2 and Figures 1-3).

3.1. Petiole

The anatomy of the petiole showed that the number of vascular bundles in the distal, median and proximal portions varied 7 to 9 (Figure 1 and Table 1). *Citrullus lanatus* had 9 vascular bundles at the distal part, 7 at the median portion and 7 at the proximal portion; *Cucumeropsis mannii* had 9 vascular bundles at the distal part, 9 at the median portion and 7 at the proximal portion while *Citrullus colocynthis* had 9 vascular bundles each at the distal, median and proximal portion (Figure 1).

3.2. Midrib

In the present study, we noted variations in the midribs of the species studied which are of taxonomic importance. The number and arrangement of the vascular are discriminating among the species studied. *C. colocynthis* had 7-vascular bundles (open crescent of 5 separate bundles with 2-accessory bundles at the adaxial surface) Figure 2A. In *C. lanatus*, the midrib had 3-vascular bundles in which 2 are parallel or superimposed and 1-accessory bundle at the adaxial surface (Table 1 and Figure 2D) and *C. mannii* had 5-vacular bundles (3 adxial separate vascular bundles in a crescent with abaxial strand between the ends of the crescent and 1-accessory bundle at the adaxial portion) (Figure 2G and Table 1).

3.3. Leaf Lamina

The adaxial and abaxial epidermal layers are made up of single layer of cells (Table 1, Figure 2 and Appendix II). The adaxial epidermal cells of *C. colocynthis* and *C. mannii* are periclinally elongated. The palisade mesophyll layers comprised 1-layer of cells in *C. mannii* and *C. lanatus* and 2-layers of cells in *C. colocynthis.* The mesophyll cells are shortened in *C. colocynthis* and *C. mannii* while in *C. lanatus*, they are anticlinally elongated (Figures 2B, E and H). The layers of spongy mesophyll varied from one species to another. These include *C. colocynthis* (3-4 layers) *C. mannii* (6 layers) and *C. lanatus* (5 layers).

3.4. Stem

The stem of *C. lanatus* had 11-vascular bundles, *C. colocynthis* and *C. mannii* 10 vascular bundles each (Figure 2). The thickness of the parenchymatous cells are 4-6 layers, 3-8 layers and 2-7 layers for *C. lanatus*, *C. colocynthis* and *C. mannii* respectively. The sclerenchymatous cell formed continuous/partly interrupted layer of cells in all the species while their thickness include 3-4 layers in *C. lanatus* and *C. mannii*, and 2-4 layers in *C. colocynthis* (Table 1).

3.5. Female Flower Stalk

The number of vascular bundles in the female flower stalk of all the species studied is ten. However, the nature and thickness of the sclerenchymatous and parenchymatous cells varied slightly among them (Figures 3A, D and G). For instance, in *C. lanatus* and *C. colocynthis*, the sclerenchymatous cells are continuous but are interrupted in *C. mannii*. In contrast, the thickness/layer of cells include 3-4 layers in *C. lanatus*, 2-7 layers in *C. colocynthis* and 3-5 layers in *C. mannii* (Figure 3 and Table 2). Also, the thickness of parenchymatous cells include 11-14 layers in *C. lanatus*, 8-12 layers in *C. colocynthis* and 6-9 layers in *C. mannii* (Figures 3, Table 2 and Appendix III).

3.6. Male Flower Stalk

In the male flower stalk, the number and nature of vascular bundles were found to be diagnostic. The number of vascular bundles in *C. lanatus* is 9, *C. colocynthis* 10 and *C. mannii* 11 (Figure 3). The sclerenchymatous cells are continuous in all the species but slightly differed in thickness. In *C. lanatus* (3-4 layers), *C. colocynthis* (2-7 layers) and *C. mannii* (4-7 layers). In the same vein the thickness/layers of parenchymatous include *C. lanatus* (1-3 layers), *C. colocynthis* (5-7 layers) and *C. mannii* (2-4 layers) Appendix III.

3.7. Tendril

The anatomical characters of the tendrils are fairly similar. For example, the sclerenchymatous cells are continuous in all the species studied. Also, the thickness of the sclerenchymatous and parenchymatous are as follows; *C. lanatus* (3-4 layers and 3-6 layers), *C. colocynthis* (3-6 layers and 4-7 layers) and *C. mannii* (4-5 layers and 5-8 layers), respectively (Figures 3C, F and I; Table 2 and Appendix III).

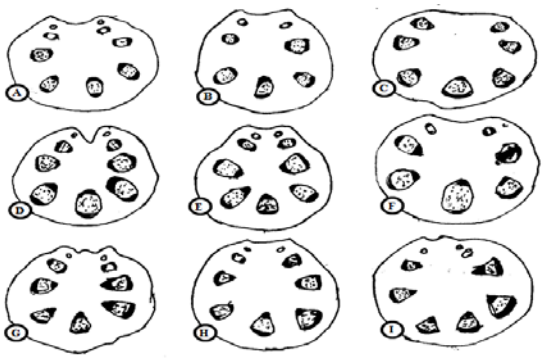

Figure 1. Sketch (Drawing) for transverse section of distal (A, D, G), median (B, E, H) and proximal (C, F, I) of the petiole. (A-C) *Citrullus lanatus*; (D-F) *Cucumeropsis mannii* and (G-I) *Citrullus colocynthis*

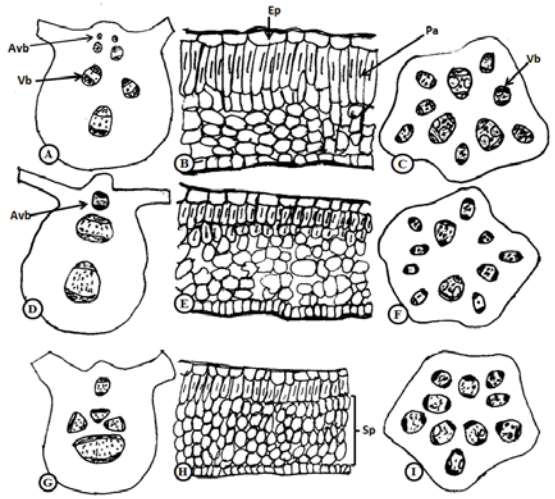

Figure 2. Sketch for anatomical sections of midrib (A, D, G), leaf lamina (B, E, H) and stem (C, F, I). (A-C) *Citrullus colocynthis*; (D-F) *Citrulus lanatus* and (G-I) *Cucumeropsis* (Avb = accessory vascular bundle, Sp = spongy mesophyll, Vb = vacular bundle, Ep = epidermal cell)

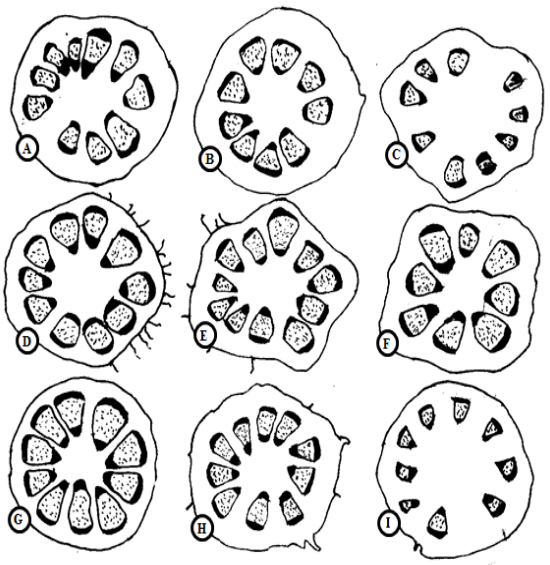

Figure 3. Sketch for transverse section of the female flower stalks (A, C, E), male flower stalks (B, D, F) and tendril (C, F, I). (A-C) *Citrullus lanatus*; (D-F) *Cucumeropsis mannii* and (G-I) *Citrullus colocynthis*

Table 1. Summary of the anatomical features of the petiole, midrib and stem of the cucurbits studied

Plant part	Features	Species name		
		***Citrullus lanatus* (Thunb.) Matsum. & Nakai.**	***Citrullus colocynthis* (L.) Schard.**	***Cucumeropsis mannii* Naud.**
Petiole	Number of vascular bundles	9 at the distal part, 7 at the median portion and 7 at the proximal portion	9 at the distal part, 9 at the median portion and 7 at the proximal portion	9 at the distal part, 9 at the median portion and 9 at the proximal portion
Midrib	Number of vascular bundles	3	7	5
	Nature/arrangement of vascular bundle	Exhibits 2 parallel or superimposed vascular bundles with 1-accessory bundle at the adaxial surface	Exhibits open crescent of 5 separate bundles with 2-accessory bundles at the adaxial surface	Exhibits adaxial crescent of 3 separate vascular bundles with abaxial strand between the ends of the crescent and 1-accessory bundle at the adaxial portion
Leaf lamina	Nature of leaf lamina	1-layer of enlongated palisade mesophyll and 3-4 layers of spongy mesophyll	2-layer of short palisade layer and 5-layers of spongy mesophyll	1-layer of short palisade layer and 5-layers of spongy mesophyll
	Nature of adaxial epidermis	Single layer	Single layer but periclinally elongated	Single layer but periclinally elongated
Stem	Number of vascular bundles	11	10	10
	Parenchymatous cell thickness	4-6 layers	3-8 layers	2-7 layers
	Nature and thickness of sclerechymatous cells	Continuous and partly interrupted, 3-4 layers	Continuous and partly interrupted, 2-4 layers	Continuous and partly interrupted, 3-4 layers

Table 2. Summary of the anatomical features of the flower stalk and tendril of the cucurbits studied

Plant part	Features	Species name		
		Citrullus lanatus	***Citrullus colocynthis***	***Cucumeropsis mannii***
Female flower stalk	Number of vascular bundles	10	10	10
	Nature and thickness of sclerenchymatous cells	Continuous with 3-4 layers	Continuous with 2-7 layers	Interrupted with 3-5 layers
	Thickness of parenchymatous cells	11-14 layers	8-12 layers	6-9 layers
Male flower stalk	Number of vascular bundles	9	10	11
	Nature and thickness of sclerenchymatous cells	Continuous with 3-4 layers	Continuous with 1-3 layers	Continuous with 2-4 layers
	Thickness of parenchymatous cells	1-3 layers	5-7 layers	4-7 layers
Tendril	Number of vascular bundles	9	9	8
	Nature and thickness of sclerenchymatous cells	Continuous with 3-4 layers	Continuous with 3-6 layers	Continuous with 4-5 layers
	Thickness of parenchymatous cells	3-6 layers	4-7 layers	5-8 layers

4. Discussion

Anatomical line of evidence has been used in classifying different plant taxa (Metcalfe and Chalk, 1979; Aguoru and Okoli, 2008; Ekeke and Agbagwa, 2016; Ekeke, *et al.,* 2016; Ekeke and Mensah, 2015; Agogbua *et al.*, 2015; Ekeke *et al.*, 2015; Ajuru and Okoli, 2013; Aguoru and Okoli, 2012). The species investigated showed close affinity based on the anatomical characteristics of their stems. In addition, the vascular bundles in all the species are of the same type (bicollateral vascular bundles). The thickness of the sclerenchymatous cells in the stems varied from 2-4 layers and was continuous or partly interrupted in all the species studied. The thickness of parechymatous cells and the number of vascular bundles varied slightly and included *C. lanatus* (4-6 layers and 11), *C. colocynthis* (3-8 layers and 10) and *C. mannii* (2-7 layers and 10), respectively. The variation in the number of vascular bundles at the distal, median and proximal portions of the petiole could be a fairly diagnostic feature among the species studied. For instance, all the species had 9-vascular bundles at the distal portion of the petiole. This indicates that they are closely related and supports their placement in one family (Hutchinson and Dalziel, 1954; Jerffery, 1990, 2005). At the median portion, *C. mannii* had 9-vascular bundles while *C. lanatus* and *C. colocynthis* had 7-vascular bundles. On the other hand, at the proximal portion, *C. colocynthis* had 7-vascular bundles while *C. mannii* and *C. lanatus* had 9-vascular bundles each. The number of vascular at these points confirmed the placement of *C. lanatus* and *C. colocynthis* in this same genus. The midrib and lamina anatomy of these species showed clear variations in the layers of mesophyll, number and arrangement of vascular bundles which are of taxonomic significance and are reported for the first time (Table 1). In the midrib, *C. lanatun* had 3-vascular (2 parallel or superimposed vascular bundles with 1-accessory bundle at the adaxial surface), *C. colocynthis* had 7-vascular bundle (open crescent of 5 separate bundles with 2-accessory bundles at the adaxial surface) while *C. mannii* had 5-vascular bundles (adaxial crescent of 3 separate vascular bundles with abaxial strand between the ends of the crescent and 1-accessory bundle at the adaxial portion). The thickness or the layers of lamina among the species were distinct. For instance, *C. lanatus* had 3-4 layers of cells, *C. colocynthis* had 6-layer of cells and *C. mannii* 5-layer of cells.

A dichomotous taxonomic key based on the anatomical characters of the species studied in the present work is presented below:

1. Petiole with 9-vascular bundles at the distal portion and 7-vascular bundles at the proximal portion; sclerechymatous cells in female flower continuous; tendril with 9-vacular bundles ---------- 2

1' Petiole with 9-vascular bundles at both the distal, median and proximal portions; sclerechymatous cells in female flower interupted; tendril with 8-vacular bundles; midrib with 5-vascular bundles; male flower stalk with 11-vascular bundles ……*Cucumeropsis mannii*

2. Midrib with 3-vascular bundles (2-parallel or superimposed vascular bundles and 1-accessory bundle at the adaxial surface); stem with 11-vascular bundles; 1-layer of palisade mesophyll; male flower stalk with 9-vascular bundles ……*Citrullus lanatus*

2' Midrib with 3-vascular bundles (5 separate or open crescent bundles and 2-accessory bundles at the adaxial surface); stem with 10-vascular bundles; 2-layers of palisade mesophyll; male flower stalk with 10-vascular bundles , ……*Citrullus colocynthis*

5. Conclusion

These anatomical features suggest taxonomic affinity amongst these species and enhance the delimitation of the species. They also strengthen the fact that the species should be maintained as separate species (Hutchison and Dalziel, 1954; Jeffrey, 1964 and 1980) and confirm the fact that the anatomical characteristics of the stem, petiole, flower stalks, midrib and leaf lamina is an important line of evidence in classification of these species.

References

Agogbua J, Chimezie, E and Bosa B E. 2015. Morpho-anatomical characters of *Zehneria capillacea* (Schumach) C. Jeffrey and *Zehneria scabra* (L.F.) Sond Cucurbitaceae. *African J Plant Sci*., **9(12):** 457-465.

Aguoru C U and Ogaba J O. 2010. Ethonobotanical survey of anti-typhoid plants amongst the Idoma people of Nigeria. *Int. Sci. Res. J.* **2**: 34-40.

Aguoru, CU and Okoli BE. 2008. Seed coat anatomy of *Momordica L.* (Cucurbitaceae) in parts of tropical western Africa. *Int. J. Trop. Agric. Food Syst*. **2(1):** 29-33.

Aguoru C U and Okoli B E. 2012. Comparative stem and petiole anatomy of West African species of *Momordica* L (Cucurbitaceae). *African J Plant Sci.,* **6(15)**, 403-409, DOI: 10.5897/AJPS11.309.

Ajuru M G and Okoli B E. 2013. The morphological characterization of the melon species in the Family Cucurbitaceae Juss. and their utilization in Nigeria. *Inter J Modern Bot.,* **3(2)**: 15-19

Burkill H M. 1985. **The Useful Plants of West Tropical Africa**. 2nd Ed. Vol. 1. Families A-D. Kew, UK: Royal Botanical Gardens, p.960.

Ekeke C and Agbagwa I O. 2016. Anatomical characteristics of Nigerian variants of *Caladium bicolor* (Aiton) Vent. (Araceae). *African J Plant Sci.,* **10(7):** 121-129.

Ekeke C and Mensah S I. 2016. Comparative anatomy of midrib and its significance in the taxonomy of the Family Asteraceae from Nigeria. *J Plant Sci.,* **10(5):** 200-205.

Ekeke C, Agogbua J and Okoli B E. 2015. Comparative anatomy of tendril and fruit stalk in Curcubitaceae Juss. from Nigeria. *Int. J. Biol. Chem. Sci.* **9(4):** 1875-1887.

Ekeke C, Ogazie C A and Mensah S I. 2016. Importance of leaf, ltem and flower stalk anatomical characters in the identification of Emilia Cass. *Inter J Plant & Soil Sci.,* **12(6):** 1-12.

Hutchinson J and Dalziel J M. 1954. **Flora of West Tropical Africa**. Crown Agents, London UK.

Hutchinson, J and Dalziel J M. 1952. **Flora of West Tropical Africa.** Vol.1. Second Edition revised by Keay, R. W. J.

Jansen van Rensburg W S, Venter S L, Netshiluvhi T R, van den Heever E, Vorster H J and de Ronde J A. 2004. Role of indigenous leafy vegetables in combating hunger and malnutrition. *South Afr. J. Bot.* **70(1):**52-59.

Jeffrey C. 1964. Key to the Cucurbitaceae of west tropical Africa with a guide to localities and little know species. *J W Afr Sci Assoc*., **9**: 79- 97.

Jeffrey C. 1980. A review of Cucurbitaceae. *Bot. J. Linn*. Soc. **81**: 233-2479.

Jeffrey C. 1990. An outline classification of the Cucurbitaceae. In: Bates DM, Robinson RW, Jeffrey C (Eds.), **Biology and Utilization of the Cucurbitaceae**. Cornell University Press Ithaca, NY. pp. 449-463.

Jeffrey C. 2005. A new system of Cucurbitaceae. *Bot. Zhurn* **90**: 3332-3335.

Metcalfe C R. and Chalk L. 1979. **Anatomy of the Dicotyledons**. 2nd Ed., Clarendon Press, Oxford, UK. P 276.

Ndukwu B C and Okoli B E. 1992. Studies on Nigerian *Curcurbita moschata. Nigerian J of Bot.*, **5**:18-26.

Okoli B E. 1984. Wild and cultivated cucurbits in Nigeria. *Economic Bot.*, **38**: 350-357.

Yamaguchi M. 1983.World Vegetables: Principles, Production and Nutritive values: West port, Conn.

Appendix I

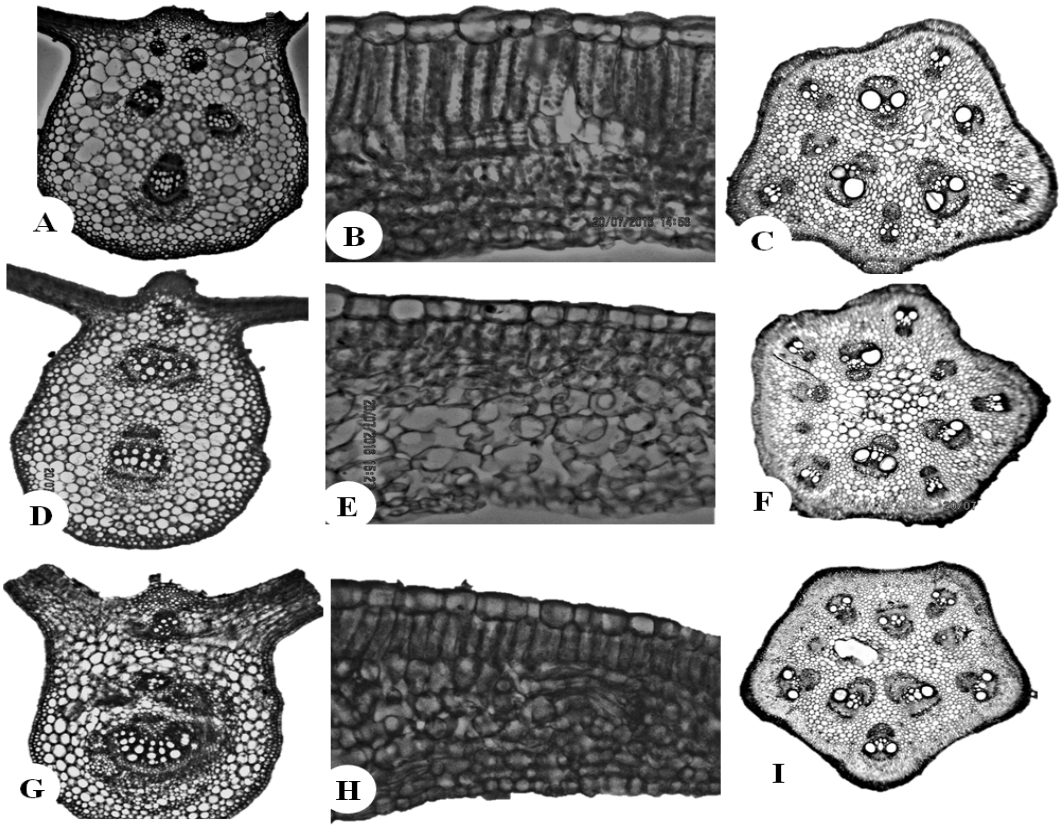

Figure 1. Anatomical sections of midrib (A, D, G), leaf lamina (B, E, H) and stem (C, F, I). (A-C) *Citrullus colocynthis*; (D-F) *Citrulus lanatus* and (G-I) *Cucumeropsis.*

Appendix II

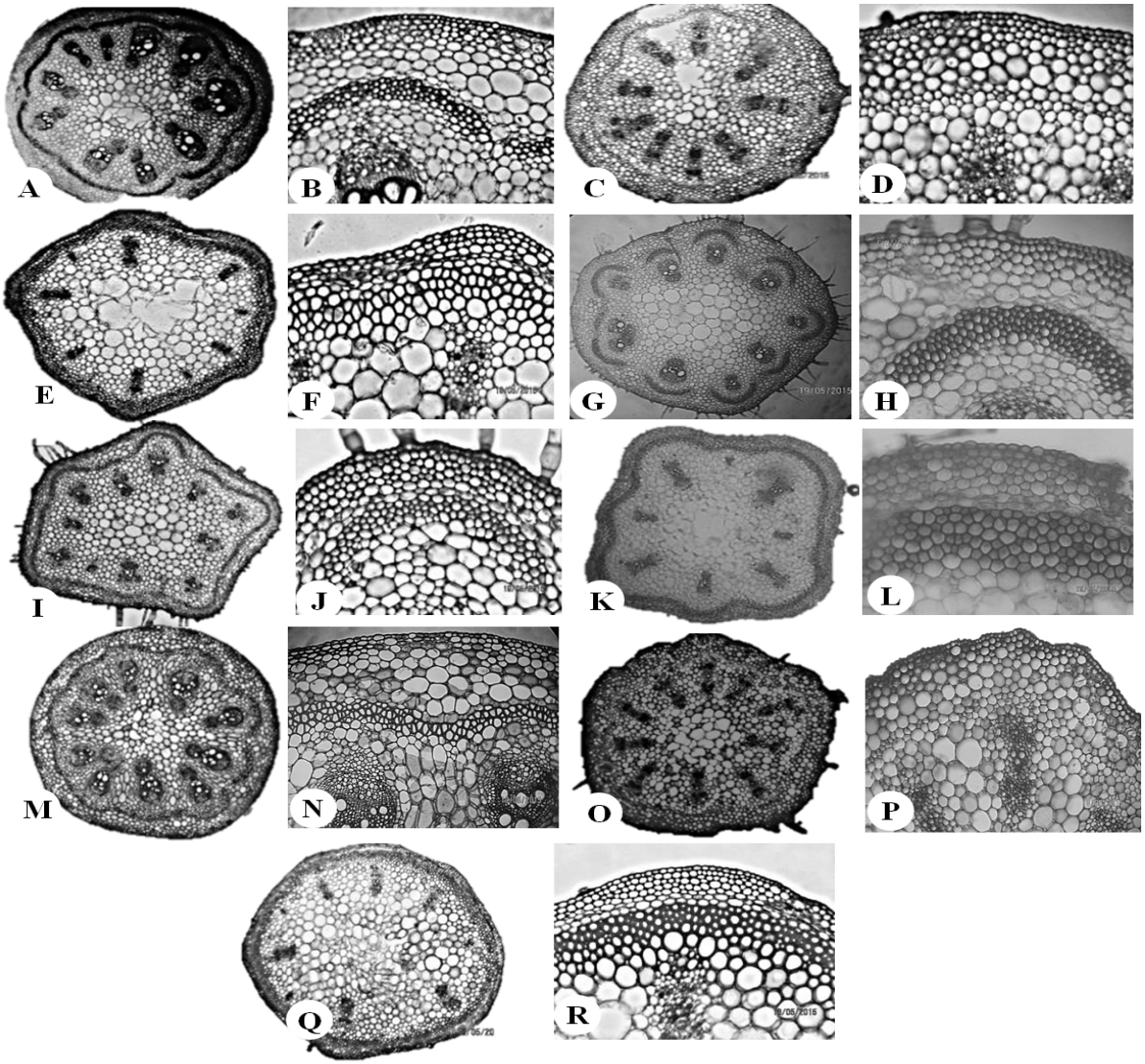

Figure 2. Transverse section of the female flower stalks (A, C, E), male flower stalks (B, D, F) and tendril (C, F, I). (A-C) *Citrullus lanatus*; (D-F) *Cucumeropsis mannii* and (G-I) *Citrullus colocynthis.* It would be better to rearrange the section photos for each species in a sequence manner for the three studied species.

Appendix III

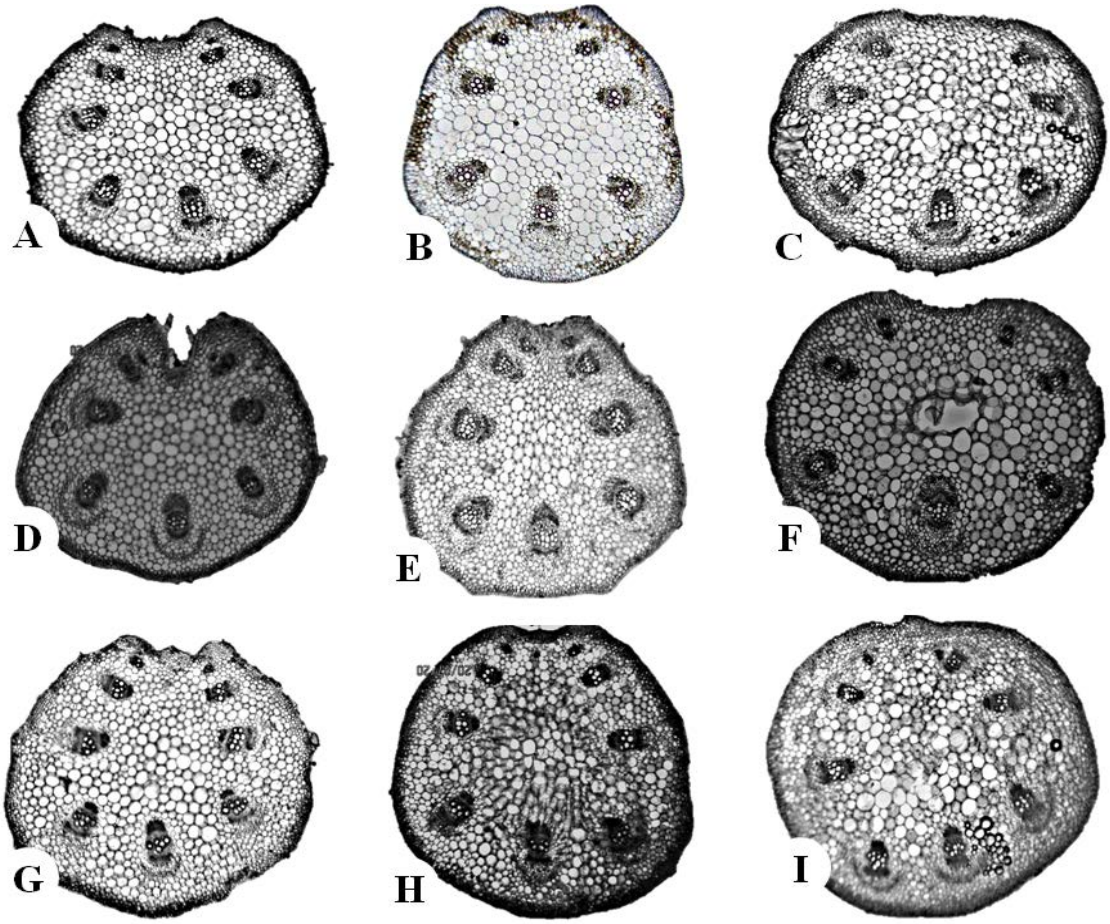

Figure 3: Transverse section of distal (A, D, G), median (B, E, H) and proximal (C, F, I) of the petiole. (A-C) *Citrullus lanatus*; (D-F) *Cucumeropsis mannii* and (G-I) *Citrullus colocyhthis.*