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COMPARATIVE ANATOMY OF *MOMORDICA DIOICA* ROXB. *EX* WILLD. AND *M. COCHINCHINENSIS* (LOUR.) SPRENG.

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Abstract

Comparative study of *Momordica dioica* Roxb. *ex* Willd. and *M. cochinchinensis* (Lour.) Spreng. based on anatomical features is carried out since such work could better describe their systematic and taxonomic significance. Patches of sclerenchyma cells in the root cortex is more in *M. cochinchinensis* than in *M. dioica.* However, stem anatomy of both the species are remarkably different in terms of number of bicollateral vascular bundles, sclerenchyma layers and contour. Presence of globular and sessile cystoliths in the lower epidermis of *M. cochinchinensis* leaves and glandular trichomes with spherical head on the adaxial ridge of *M. cochinchinensis* midrib are distinct characters to differentiate with *M. dioica.* Petiolar anatomy reveales further disparate natures with trichome and contour though both the species have similar number of bicollateral vascular bundles and raphides in the ground tissue. A dichotomous key on the studied species is prepared based on anatomical features.

Introduction

The genus *Momordica* belongs to the tribe Joliffieae (Schrad.) of Cucurbitaceae and is a native of the Palaeotropics consisting of about 40 species distributed in warm tropics of both hemispheres, chiefly in Africa and with about 10 species in South-East Asia (Chakravarty 1982, De Wilde and Duyfjes 2002). Ahmed *et al.* (2008) reported three species of *Momordica* from Bangladesh, namely *Momordica charantia* L., *M. cochinchinensis* (Lour.) Spreng. and *M. dioica* Roxb. *ex* Willd. In recent years, teasle gourd *i.e. M. dioica* (locally known as Kakrol) has become a popular vegetable due to its good nutritional value and ubiquitous distribution nature. *M. dioica* is a perennial, climbing herb with tuberous roots. This plant is characterized by presence of slender stem, filiform tendrils, ovate-cordate, entire or variously lobed leaves with eglandular petioles, dioecious in nature and fruits are ovate or broadly ovate, spinescent (Ahmed *et al.* 2008). *M. dioica* contains good amount of carotene (160 mg/100 gm) and protein (3.1 gm/100 gm) amongst all Cucurbitaceous vegetables (Baratakke *et al.* 2013). The mucilaginous tuber is used against bleeding of piles and a sedative in fever. It is also used as an expectorant. Moreover, anti-dibetic, anti-lipid peroxidative (Ilango *et al.* 2009), anti-inflammatory, antioxidant and hepato-protective activities have also been reported (Shreedhara and Vaidya 2006).

M. cochinchinensis commonly known as giant spine gourd (local name: Golkakra, Bonkakrol) is a perennial, climbing herb with tuberous roots featuring robust, angular stem with robust tendrils and broadly sub orbicular, lanceolate leaves where petioles are robust, glandular, dioecious and fruits are fleshy, ovoid, acute and densely aculeate (Ahmed *et al.* 2008). The whole plant is considered stomachic and stimulant and is used in cough and seeds are used as laxative and in the treatment of ulcers, sores and obstruction of liver and spleen whereas fruits and leaves offer external applications in lumbago and ulceration (Ahmed *et al.* 2008). This plant has been shown to contain up to 70 times the amounts of lycopene found in tomatoes and 10 times the amount of beta-carotene of carrots or sweet potatoes (Ishida *et al.* 2004). Besides, anticancerous and antioxidative properties have been reported (Kubola and Siriamornpun 2011, Tien *et al.* 2005).

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Comparative or systematic plant anatomy which introduces the comparative study of the representatives of different species, genera, families and has been used for clarification along with better understanding of their phylogenetic bonds. Extensive anatomical investigations have been carried out in *M. charantia* (Yasuda 1903, Aguoru and Okoli 2012, Giuliani *et al.* 2016, Poyraz and Derdovoski 2016). Though several anatomical studies have been carried out on *M. dioica* and *M. cochinchinensis* (Sonker *et al.* 2014, Lan Huong and Ba T 2012), no detailed comparative works on these two species have been reported so far. The main objective of the present study is to investigate detailed anatomical features of the root, stem, leaf and petiole in two species of *Momordica*, namely *M. dioica* and *M. cochinchinenesis* by using light microscopy.

Materials and Methods

Teasle gourd (*Momordica dioica* Roxb. *ex* Willd.) and giant spiny gourd (*M. cochinchinensis* (Lour.) Spreng.) were used as experimental materials. Fresh specimens were collected from Dhaka University Botanical garden, Department of Botany. Information regarding voucher specimen of the materials are as follows:

M. cochinchinensis (Lour.) Spreng., collection area Dumki, Krishi University area, collection date-17.05.2005, collected by - M. Sultana, voucher specimen no.- DUSH872.

M. dioica Roxb. *ex* Willd., collection area - Dhaka University area, collection date-15.10.2015, collected by - K.J. Shethi, voucher specimen no.- DUSH1093.

For anatomical study root, stem, leaf and petiole were collected from the Botanical Garden, Department of Botany, University of Dhaka. Free hand sectioning of root, stem and leaf was done with the help of a razor blade. The sections were stained in safranin, mounted in 20% glycerol and studied with the help of a light microscope (Carl Zeiss Lab.A1 microscope). Micrographs of the sections were taken using Axiocam ERc 5s digital camera attached with computer through Axio Vision Release 4.8.2 software.

Results and Discussion

Transverse sections of roots of *Momordica dioica* and *M. cochinchinensis* are shown in Fig. 1a, b and vascular bundles of both the species are shown in Fig. 1c, d. Since both the species are perennial periderm is observed from root sections though it is not so well developed in *M. dioica* as in *M. cochinchinensis*. The contour of root is not diversified as that of the stem and is roundish for both the species. Numerous masses of sclerenchyma are found scattered in the cortex outside the vascular bundles as well as surrounds the vascular bundles in *M. dioica* (Fig. 1a). In *M. cochinchinensis* sclerenchyma cells are present in 3 - 4 tangential parallel rows in the cortex (Fig. 1b) and vascular bundles are also covered with thick patches of sclerenchyma cells. For both the species vascular bundles is tetrarch. The xylem vessels in the root of *M. dioica* are larger than that of *M. cochinchinensis* (Fig. 1c, d).

Stem sections of both the species show ridges and furrows which is a feature of Cucurbitaceae. The transverse section of stem reveals that the stem of *M. dioica* has typical five angles or ridges with more or less a star like outer-structure (Fig. 2a), whereas *M. cochinchinensis* exhibits five ridges but they are not so prominent as *M. dioica* and shows somewhat wavy outlook (Fig. 2b). The epidermal layer of *M. dioica* has single layer of parenchymatous cells which are hexagonal and has thin cuticle layer. Collenchyma cells (hypodermis) lie just beneath the epidermis and consists of many layers of cells in the ridges, whereas in the furrows it is a few layered (Fig. 2e). Two layers of chlorenchyma cells are present just below the collenchyma in case of the ridges but not in the furrows (Fig. 2e). The stem is characterized by pericycle of continuous ring of a few layered sclerenchyma cells (Fig. 2c). Five bicolatteral and open vascular

bundles are situated in a single ring in the centre. Each of the five angles has one fibro-vascular bundle where xylem is surrounded by inner and outer phloem (Fig. 2e). Metaxylems of the central vascular bundles are larger than the fibro-vascular bundles situated at the angles (Fig. 2a).

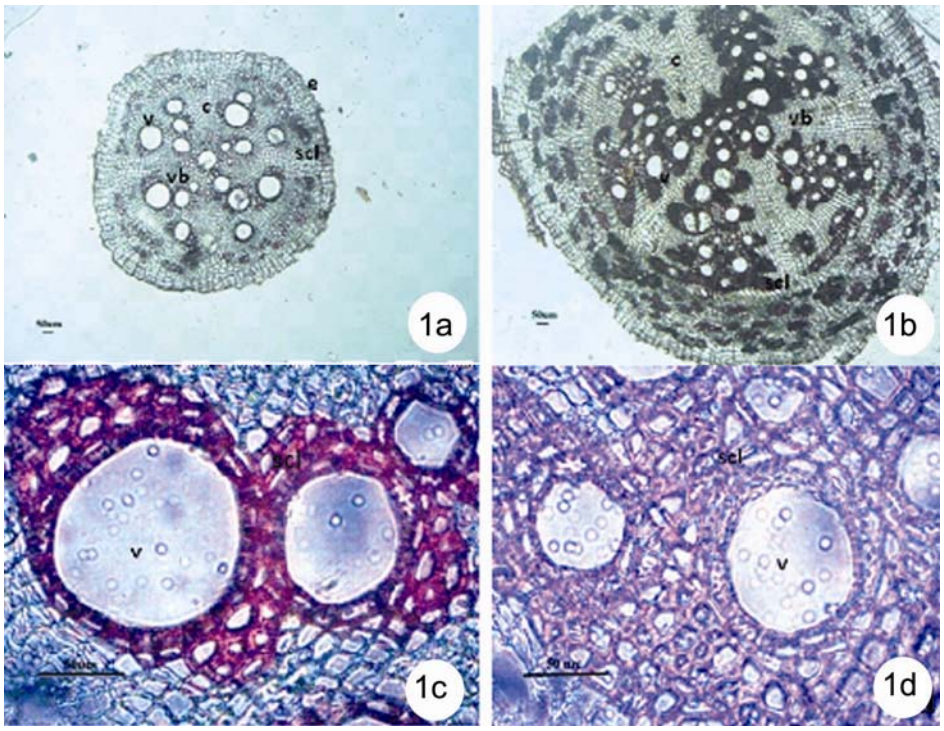

Fig.1. Transverse sections of root of *M. dioica* (a) and of *M. cochinchinensis* (b) under 50X magnification. Xylem cells of *M. dioica* is larger in diameter (c) and comparatively small in *M. cochinchinensis* (d) under higher magnification (400X). e = epidermis, scl = sclerenchyma, vb = vascular bundle, v = vessel. Bar = 50 µm.

Fig. 2b presents the transverse section of *M. cochinchinenesis* stem. Epidermis is single layered with quadrangular cells and covered with thick cuticle layer. Multilayered (8 - 10) collenchymatous hypodermis is observed below the epidermis (Fig. 2d). Following the hypodermis a few layers of chlorenchymatous cells are present and beneath this continuous ring of pericycle are present with multilayered sclerenchymatous cells (Fig. 2f). Each of the five ridges has three fibro-vascular bundles with a total of 15 fibro-vascular bundles (Fig. 2b) (Lan and Ba 2011) contrary to that of *M. dioica* (Fig 2a). Besides these vascular bundles, five well developed bicolatteral vascular bundles are arranged in a ring in the center of cortex. Metaxylems of the central vascular bundles of *M. cochinchinensis* are prominent and large comparing to that of *M. dioica* (Fig. 2a, b). Pith is present in both the species.

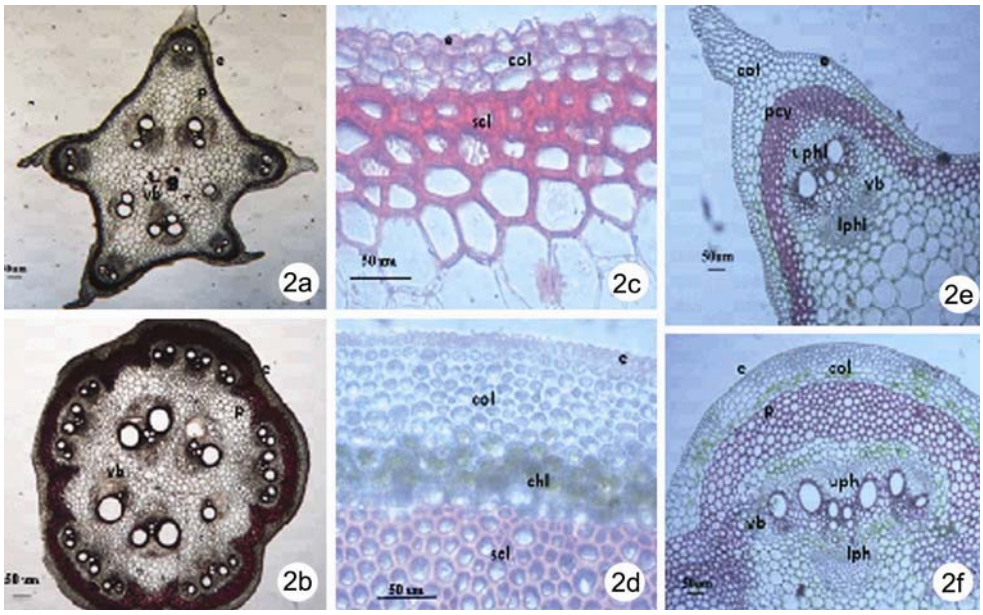

Fig. 2. Transverse sections of stem of *M. dioica* (a) and *M. cochinchinensis* (b) under 20X magnification. Epidermis is followed by collenchymas and sclerenchyma cell layers in *M. dioica* (c) and for *M. cochinchinensis* epidermis followed by collenchyma, chlorenchyma and sclerenchyma cell layers (d) under 400X magnification. Single fibro-vascular bundle at one of the ridges of *M. dioica* (e); on the contrary three fibro vascular bundles at one of the ridges of *M. cochinchinensis* (f) 100X magnification. chl = chlorenchyma, col = collenchyma, e = epidermis, p = pericycle, scl = sclerenchyma, uph = upper phloem, lph = lower phloem, vb = vascular bundle. Bar = 50 µm.

Leaf transverse sections in *M. dioica* and *M. cochinchinensis* appear different from each other. The midrib portion is bulged towards the abaxial side of the leaf in *M. dioica* (Fig. 3a), while for *M. cochinchinensis* midrib is bulged towards the abaxial side and projected as angle towards the adaxial side (Fig. 3b). Upper epidermis and lower epidermis of both the species is uniseriate having barrel shaped cells followed by a few layers of well developed collenchyma cells only at the lower margins. Multilayered chlorenchyma cells are present below the collenchyma cells in the midrib of the two species (Fig. 3c, d). The central portion of the midrib occupied by well developed vascular bundle in *M. dioica*. However, in *M. cochinchinensis* two/three more inconspicuous vascular bundles in addition to a well developed vascular bundle are present on the adaxial side (Fig. 3d). Our finding is in conformity with the work of Lan Huong and Ba T 2012. Glandular trichomes with uniseriate stalks and spherical or disc-shaped head is present on the adaxial ridge of *M. cochinchinensis* midrib which was also indicated by Metcalfe and Chalk (1950) in this species along with other species of *Momordica*. Upper epidermis and lower epidermis cells of the lamina of both the species are wavy with thick cuticle and the upper epidermal cells of *M. dioica* are elongated in shape (Fig. 3e, f). Palisade tissue consists of two layers of elongated cells and spongy tissue contains loosely arranged irregular cells in *M. dioica* (Fig. 3e). On the other hand, palisade tissue of *M. cochinchinensis* has single layer of elongated cylindrical cells beneath the upper epidermis and spongy tissue is compactly arranged with irregular cells. Lower epidermal cells have lot of double and scarcely seated stalkless globular cystoliths (Fig. 3f) which is not found in *M. dioica*.

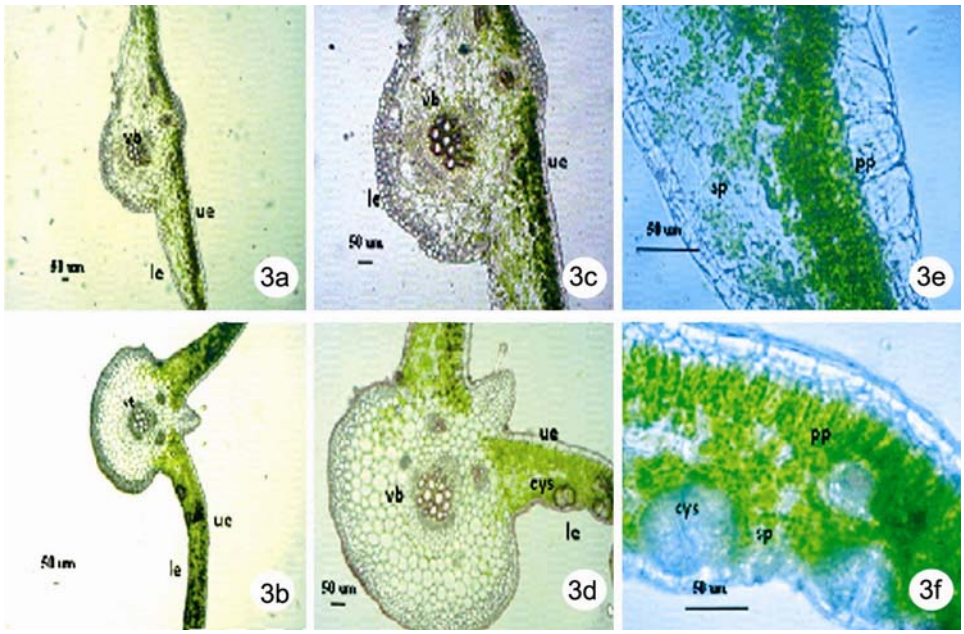

Fig. 3. Leaf transverse section of *M. dioica* (a) and *M. cochinchinensis* (b) under 50X magnification. Midrib of *M. dioica* with a vascular bundle (c), midrib of *M. cochinchinensis* showing a well developed vascular bundle along with two fibro vascular bundles towards adaxial side (d) under 100X magnification. Elongated epidermal cells on the upper surface of *M. dioica* (e); *M. cochinchinensis* is characterized by cystoliths on the lower surface (f) under 400× magnification. cys = cystolith, le = lower epidermis, pp = palisade parenchyma, sp = spongy parenchyma, ue = upper epidermis, vb = vascular bundle. Bar = 50 µm.

Petiole anatomy reveals that in *M. dioica* petiole is octagonal with eight ridges (Fig. 4a), whereas *M. cochinchinensis* is parabolic in one side with three outgrowths on other side (Fig. 4b). Epidermis of both *M. dioica* and *M. cochinchinensis* has single layer barrel shaped cells with cuticle. Four - five layers of collenchyma cells are found immediately beneath the epidermis which is followed by several layers of clorenchyma cells in *M. dioica* (Fig. 4c) but in *M. cochinchinensis* epidermis is followed by patches of sclerenchymatous cells and chlorenchyma (Fig. 4d). Each species has nine vascular bundles of various sizes where the bigger ones are towards the lower surface. Vascular bundles are bi-collateral, arranged in a ring and surrounds with layers of sclerenchyma towards the upper side. Xylem cells are many and conspicuous in *M. cochinchinensis* compared to *M. dioica* (Fig. 4e, f) (Yasuda 1903). Ground tissue is parenchymatous with starch depositions (raphides) at the corners in both the species (Fig. 5a, b). *M. dioica* has sharp pointed, multi-celled, conical shaped trichome but *M. cochinchinensis* has blunt tiped, multi-celled, hook shaped trichome (Fig. 5c, d). Comparative morphological study of *M. dioica* and *M. cochinchinensis* displays that the petiole of *M. cochinchinensis* is glandular, whereas *M. dioica* petiole is eglandular (Ahmed *et al*. 2008). Figure 5f revealed the internal structure of petiolar glands (kidney shaped) of *M. cochinchinensis* with thick cuticular epidermis followed by a few layers of collenchyma. Beneath the collenchymas, multilayered parenchymatous cells are present and a good number of cavities are embedded in these cells. Within the cavities xylem is observed.

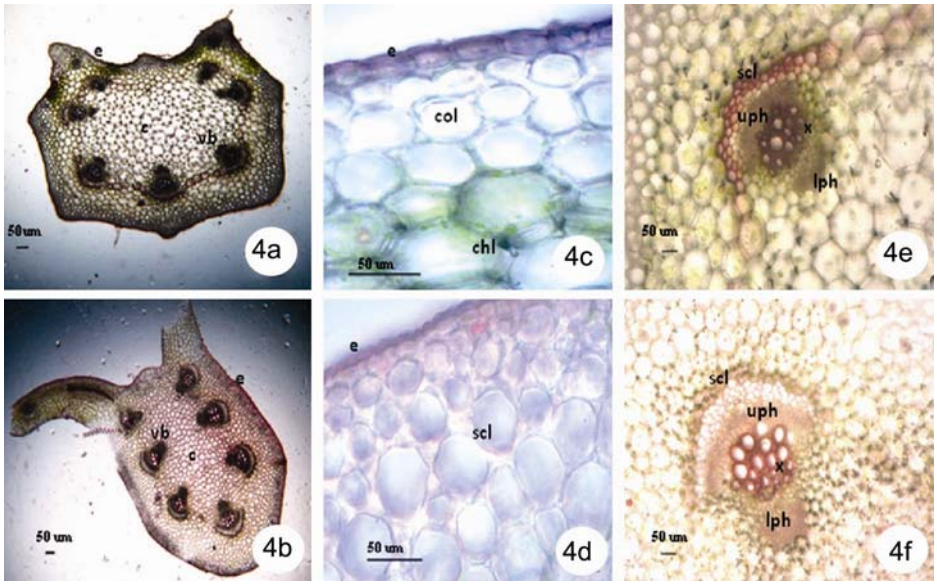

Fig. 4. Transverse section of petiole of *M. dioica* (a) and *M. cochinchinensis* (b) under 20X magnification. Epidermis with thick cuticle is followed by collenchymas cells in *M. dioica* (c) whereas sclerenchyma cells present beneath the cuticularized epidermis in *M. cochinchinensis* (d). Bi-collateral vascular bundles of *M. dioica* (e) and *M. cochinchinensis* (f) under 100× magnification. chl = chlorenchyma, col = collenchyma, c = cortex, epi = epidermis, lph = lower phloem, scl = sclerenchyma uph= upper phloem, vb = vascular bundle, x = xylem. Bar = 50 µm.

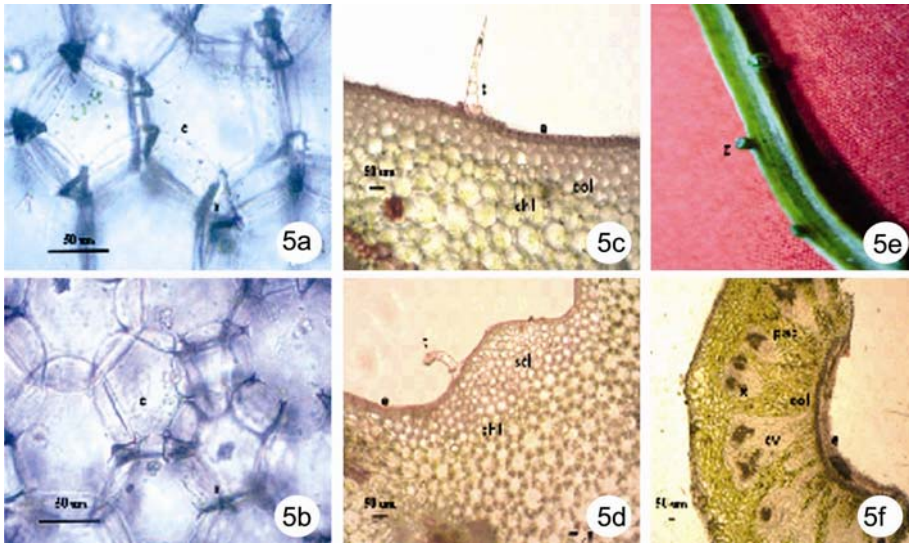

Fig. 5. Pith with raphides is observed in both species: *M. dioica* (a) and *M. cochinchinensis* (b) 400X magnification. Sharp pointed, multi-celled, conical shaped trichome on the petiole of *M. dioica* (c); trichme of *M. cochinchinensis* is blunt tipped, multi celled and hook shaped (d) 100X magnification. Glands on petiole of *M. cochinchinensis* (e). Longitudinal section of gland showing many cavities with xylem embedded in chlorophyllous parenchyma cells (f) 50× magnification. chl = chlorenchyma, col = colenchyma, c = cortex, cv = cavity, e = epidermis, pac = parenchyma, r = raphide, t = trichome, x = xylem. Bar = 50 µm.

In the present study detailed anatomical investigation *M. dioica* and *M. cochinchinensis* was conducted. These investigations are reported for the first time in Bangladesh. Well documented differences have been observed in anatomical studies of both the species. An indented taxonomic key based on root, stem, leaf and petiole anatomical characters of *M. dioica* and *M. cochinchinensis* is given below:

Number of patches of sclerenchyma in the root not more than 2; stem with 10 bi-collateral vascular bundles; cystolith absent; transverse section of petiole is octagonal in shape; in sclerenchymal cortex number of patches not more than two layers *M. dioica*

Number of patches of sclerenchyma in the root more than 2; stem with 20 bi-collateral vascular bundles; cystolith present in spongy parenchyma; transverse section of petiole is parabolic; in sclerenchymal cortex number of patches more than two layers *M. cochinchinensis*

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