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ORIGINAL ARTICLE

Taxonomic significance of trichomes micromorphology in cucurbits

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Abstract Studies on trichomes micromorphology using Scanning Electron Microscope (SEM) were undertaken in 23 species with one variety under 13 genera of the family Cucurbitaceae (viz., *Benincasa hispida* (Thunb.) Cogn., *Citrullus lanatus* (Thunb.) Matsum. & Nakai, *Cucumis melo* var. *agrestis* Naudin, *Cucumis sativus* L., *Diplocyclos palmatus* (L.) C. Jeffrey, *Edgaria darjeelingensis* C.B. Clarke, *Gynostemma burmanicum* King ex Chakr., *Gynostemma pentaphyllum* (Thunb.) Makino, *Gynostemma pubescens* (Gagnep.) C.Y. Wu, *Hemsleya dipterygia* Kuang & A.M. Lu, *Lagenaria siceraria* (Molina) Standl., *Luffa acutangula* (L.) Roxb., *Luffa cylindrica* M. Roem., *Luffa echinata* Roxb., *Melothria heterophylla* (Lour.) Cogn., *Melothria leucocarpa* (Blume) Cogn., *Melothria maderspatana* (L.) Cogn., *Sechium edule* (Jacq.) Sw., *Thladiantha cordifolia* (Blume) Cogn., *Trichosanthes cucumerina* L., *T. cucumerina* var. *anguina* (L.) Haines, *Trichosanthes dioica* Roxb., *Trichosanthes lepiniana* (Naudin) Cogn. and *T.* tricuspidata Lour.). The trichomes in the family Cucurbitaceae vary from unicellular to multicellular, conical to elongated, smooth to ridges, with or without flattened disk at base and cyctolithic appendages, thin to thick walled, curved at apices to blunt. Trichomes micromorphology in the family Cucurbitaceae was found significant taxonomically.

1. Introduction

Cucurbitaceae, with c. 800 species under 130 genera (Jeffrey, 2005) are among the economically most important plant families (Pandey et al., 2006; Ali, 2006; Ali and Pandey, 2006, 2007; Ali et al., 2009). Jeffrey (2005) divided Cucurbitaceae into 11 tribes under two subfamilies viz., the Nhandiroboideae (Zanonioideae, with 60 species under 19 genera) and Cucurbitoideae (with c. 740 species under 111 genera).

Scanning Electron Microscopy (SEM) is an ideal technique for examining plant surfaces at high resolution (Pathan et al., 2008). Micromorphology of vegetative and reproductive plant organs is the object of research to resolve the taxonomic

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problems of critical species and genera. Trichomes are distributed at the surface of aerial plant parts, having various functions and are extremely variable in their presence across plant, location on plant organs, density, form, etc., and, therefore, their morphology and structure can be used as taxonomic markers in the infrageneric classification of the genus (Haron and Moore, 1996; Wang et al., 2007; Husain et al., 1994; Rapisarda et al., 1997; Leelavathi and Ramayya, 1983; Banerjee et al., 2004; Kannabiran and Krishnamurthy, 1972).

The most important diagnostic characters for the genera and tribes of Cucurbitaceae come from androecium and gynoecium morphology, type of tendril branching, pollen structure and seed coat structure (Jeffrey, 2005). Shanta and Radhakrishaniah (2000) studied cladistic approach of some of the genera of Cucurbitaceae. Zhang et al. (2006) inferred phylogeny of Cucurbitales based on nine plastids (*atp*B, *mat*K, *ndh*F, *rbc*L, the *trn*L-F region, and the *rpl20–rps12* spacer), nuclear (18S and 26S rDNA), and mitochondrial (*nad1* b/c intron) genes. Kocyan et al. (2007) inferred phylogeny of Cucurbitaceae based on chloroplast DNA sequences from two genes, one intron and two spacers (*rbc*L, *mat*K, *trn*L, *trn*L–*trn*F, *rpl20–rps12*). A perusal of literature reveals that tri-

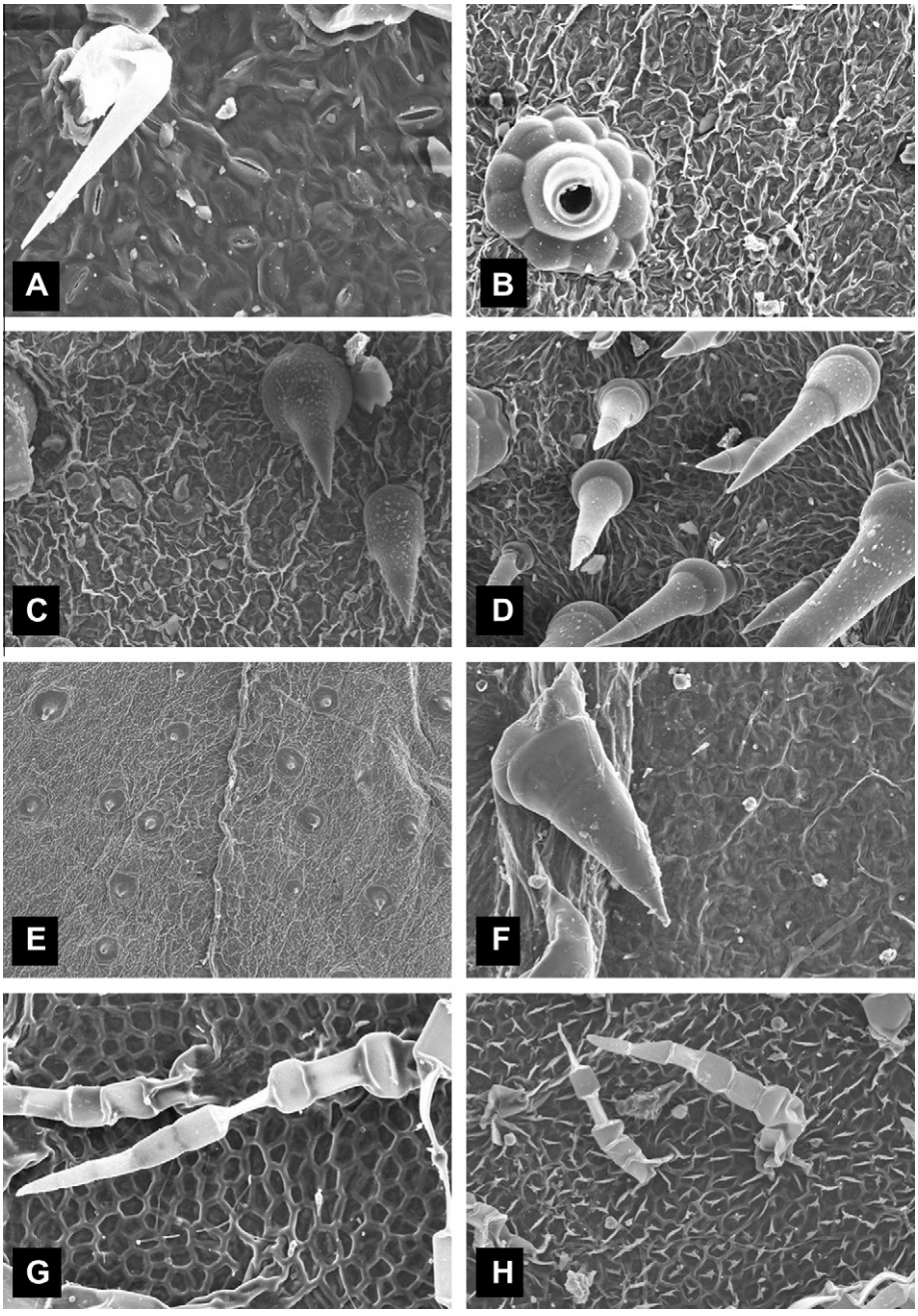

Figure 1 Trichomes morphology in Cucurbitaceae: (A) *Benincasa hispida* ×300, (B) *Citrullus lanatus* ×300, (C) *Cucumis melo* var. *agrestis* ×300, (D) *C. sativus* ×300, (E) *Diplocyclos palmatus* ×50, (F) *Edgaria dargeelingensis* ×300, (G) *Gynostemma burmanicum* ×300, and (H) *G. pentaphyllum* ×300.

chome morphology has not been studied in the systematics of Cucurbitaceae. The present study aims to survey and evaluate the trichomes micromorphology in Cucurbitaceae using SEM to provide new insights into its potential taxonomic value.

2. Materials and methods

Twenty three species with one variety under 13 genera of Cucurbitaceae (viz., *Benincasa hispida, Citrullus lanatus, Cucumis melo* var. *agrestis*, *Cucumis sativus*, *Diplocyclos palmatus, Edgaria dargeelingensis, Gynostemma burmanicum, Gynostemma pentaphyllum, Gynostemma pubescens, Hemsleya diptrygia, Lageneria siceraria, Luffa acutangula, Luffa cylindrica, Luffa echinata, Melothria heterophylla, Melothria leucocarpa, Melothria maderspatana, Sechium edulae, Thladiantha cordifolia, Trichosanthes cucumerina, T. cucumerina* var. *anguina*, *Trichosanthes dioica*, *Trichosanthes* lepiniana and *T. tricuspidata*) were scanned using Scanning Electron Microscope (SEM). Young leaves (first fully expanded leaf from the tip) and old leaves (third or fourth fully expanded leaf from the tip) were collected from each plant. Plant specimens were prepared for SEM using procedures described by McWhorter et al. (1993). Squares of leaves (with approx. 1 mm thickness of underlying tissues) were excised using a razor blade from the

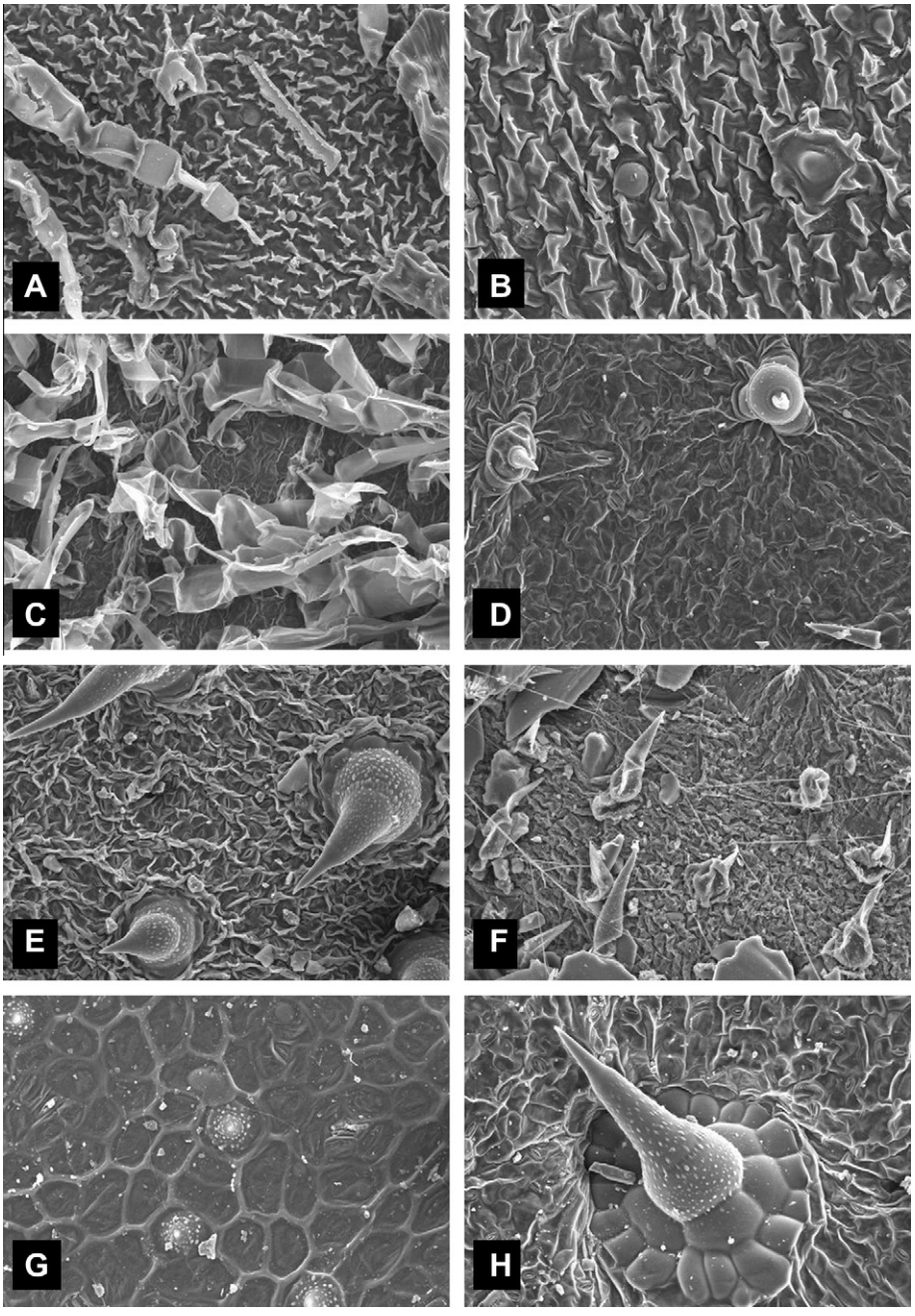

Figure 2 Trichomes morphology in Cucurbitaceae: (A) *Gynostemma pubescens* ×300, (B) *Hemsleya diptrygia* ×300, (C) *Lageneria siceraria* ×300, (D) *Luffa acutangula* ×300, (E) *L. cylindrica* ×300, (F) *L. echinata* ×300, (G) *Melothria heterophylla* ×300, and (H) *M. leucocarpa* ×300.

plant, avoiding the midrib areas so as to give a relatively consistent surface. Leaf segments of approximately 20 mm^2 were fixed for 12 h in 4% Glutaraldehyde and were rinsed three times with distilled water before dehydration in a graded ethanol series. Samples were dried in a critical point drier and were mounted on aluminum stubs using two-sided adhesive carbon tape. The samples were then coated with very thin layer of gold in a sputter coater unit (Hitachi E-1010), and observed with a Hitachi S3400-N Scanning Electron Microscope at 20 kV. Electron images were recorded using a digital image processor.

3. Results

The trichomes in Cucurbitaceae vary from unicellular to multicellular, conical to elongated, smooth to ridges, with or without flattened disk at base and cyctolithic appendages, thin to thick walled, curved at apices to blunt.

3.1. Benincasa

The trichomes in *B. hispida* are unicellular, simple, conical, swollen at base, without cystolithic appendages and ended with

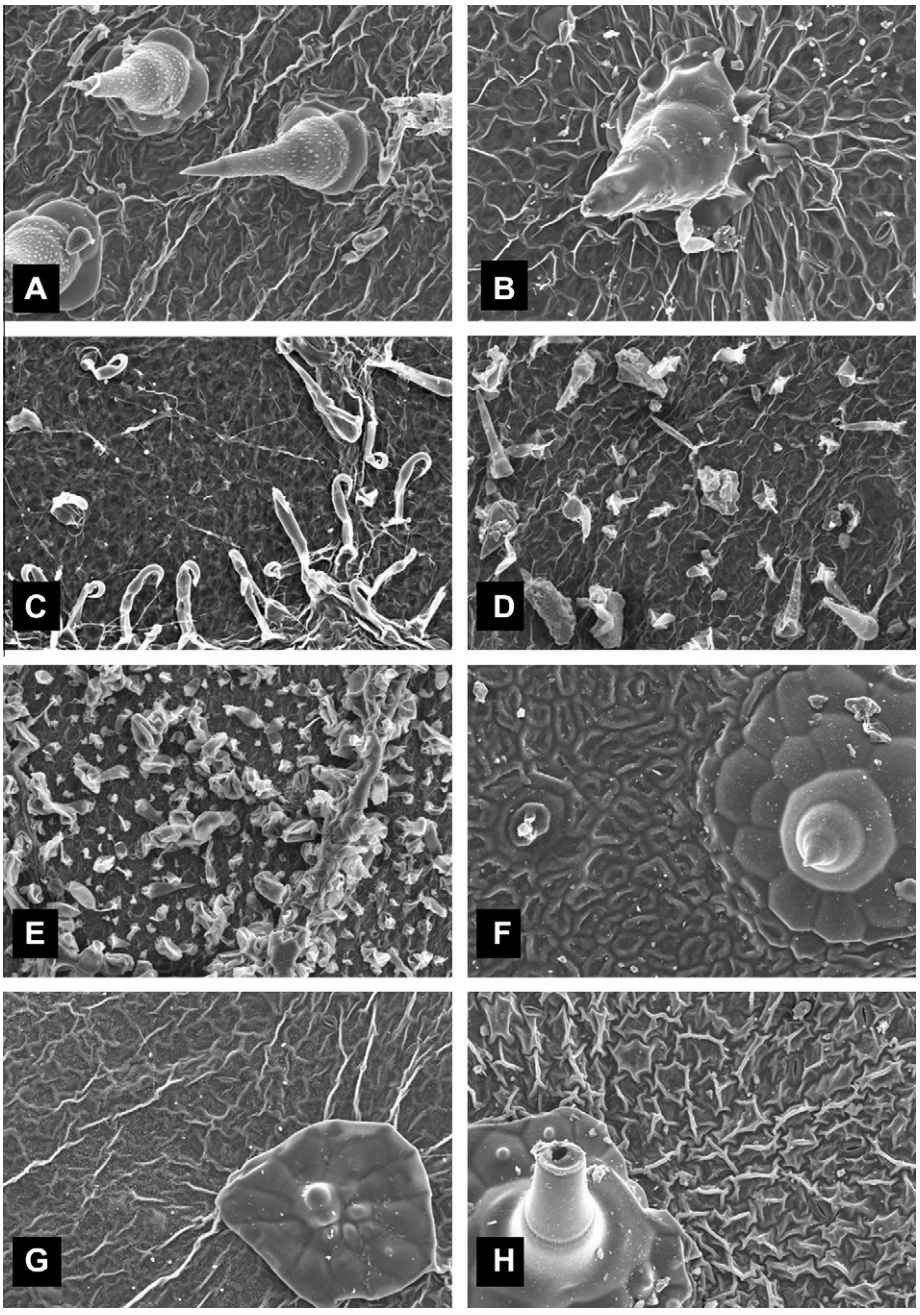

Figure 3 Trichomes morphology in Cucurbitaceae: (A) *Melothria maderspatana* ×300, (B) *Sechium edulae*, (C) *Thladiantha cordifolia* ×300, (D) *Trichosanthes cucumerina* ×300, (E) *T. cucumerina* var. *anguina* ×300, (F) *T. dioica* ×300, (G) *T. lepiniana* ×300, and (H) *T. tricuspidata* ×300.

a pointed tip. The numbers of trichomes are more at midrib. Some trichomes at mid rib position are elongated and thin walled (Fig. 1A).

3.2. Citrullus

The trichomes in *C. lanatus* are multicelluar (2–3 celled) and are without cystolithic appendages. The trichomes at mid rib position are longer than the trichomes away from the mid rib. The base of the trichomes is flattened, often curved, surrounded by 9–12 sub spheroid adjacent subsidiary cells. Both long and short trichomes ended with pointed tips (Fig. 1B).

3.3. Cucumis

The trichomes in *C. melo* var. *agrestis* are short, thick walled, conical, 2–3 celled, with cystolithic appendages, swollen at base and ended with pointed tips (Fig. 1C). In *C. sativus*, the trichomes are distributed all over the leaf surface and comparatively dense at mid rib. Some trichomes are smaller in size. The trichomes are 2–3 celled, thick walled, conical, with few cystolithic appendages, swollen at base and ended with pointed tips (Fig. 1D).

3.4. Diplocyclos

The trichomes in *D. palmatus* are few, rudimentarily developed with flattened base (Fig. 1E).

3.5. Edgaria

The trichomes in *E. dargeelingensis* are of two types i.e., smaller and larger trichomes. The larger trichomes are confined at mid rib. The trichomes are conical, swollen at base, thick walled and without cystolithic appendages (Fig. 1F).

3.6. Gynostemma

The trichomes in *G. burmanicum* are multicellular, thick walled, elongated, with transverse ridges, ended with pointed apex, with cystolithic appendages and without flattened disk at base (Fig. 1G). In *G. pentaphyllum*, the trichomes are few, multicelluar, thick walled, conical, with either acute or blunt end and without flattened disk at base (Fig. 1H). In *G. pubescence*, the trichomes are many, multicellular, thick walled, elongated, with ridges, ended with acute apex, without flattened disk at base and with cystolithic appendages (Fig. 2A).

3.7. Hemsleya

The trichomes in *H. diptrygia* are few, rudimentarily developed, represented by globular structure, without flattened disk at base and cystolithic appendages (Fig. 2B).

3.8. Lageneria

The trichomes in *L. siceraria* are dense, slightly more at mid rib, elongated, thin walled, with ridges, usually ended with acute apex and without cystolithic appendages (Fig. 2C).

3.9. Luffa

The trichomes in *L. acutangula* are rudimentarily developed, with 3–4 rudimentary developed flattened basal cells. The apical cells are small and suddenly pointed. Basal cells are with cystolithic appendages (Fig. 2D). In *L. cylindrica*, the trichomes are two-celled, with cystolithic appendages, curved pointed apical cell and 8–10 flattened basal cells (Fig. 2E). In *L. echinata*, the trichomes are acystolithic, thin walled, some what elongated and lack flattened base (Fig. 2F).

3.10. Melothria

The trichomes in *M. heterophylla* are few, rudimentarily developed with small apical cell and are with cystolithic appendages (Fig. 2G). In *M. leucocarpa*, trichomes are thick walled, conical, acute at apex, two rows of prominent flattened base (inner 5–7 celled and outer 15–20 celled) and are with cystolitic appendage (Fig. 2H). In *M. maderspatana*, trichomes are 2–3 celled, conical, acute, cystolithic and are with flattened base (3–5 celled inner rows and 5–7 celled outer rows) (Fig. 3A).

3.11. Sechium

The trichomes in *S. edulae* are conical, thick walled, 2–3 celled with rudimentarily developed flattened disk at base and ended with acute apex (Fig. 3B).

3.12. Thaladiantha

The trichomes in *T. cordifolia* are elongated, without any flattened disk, bended at apex and ended with pointed tip (Fig. 3C).

3.13. Trichosanthes

In *T. cucumerina*, trichomes are densely distributed throughout the surface, thin walled, irregular in shape and without flattened disk at base, however, some trichomes are slightly conical and end with acute apex (Fig. 3D). In *T. cucumerina* var. *anguina*, trichomes are densely distributed, thin walled, irregular in shape, without flattened disk at base, slightly blunt apex (Fig. 3E). In *T. dioica*, trichomes are 2–3 celled, with prominent flattened disk of two rows of cells (outer comprises 15–18 celled while inner comprises 7–10 celled). The trichome apex ended with pointed tip (Fig. 3F). In *T. lepiniana*, the trichomes are rudimentarily developed, with 8–10 celled flattened base and are distributed poorly over the surface (Fig. 3G). In *T. tricuspidata*, the trichomes are 2–3 celled with well developed flattened disk at base, slightly curved at end with acute apex and are without cystolithic appendages (Fig. 3H).

4. Discussion

The importance of epidermal characters of leaves in angiosperms have been reviewed by several authors (Hagerup, 1953; Stebbins and Jain, 1960; Borril, 1961; Stace, 1984; Nyawuame and Gill, 1990; Edeoga, 1991; Edeoga and Ikem, 2001; Parveen et al., 2000). The trichomes in Cucurbitaceae

vary from unicellular to multicellular, conical to elongated, smooth to ridges, with or without flattened disk at base and cyctolithic appendages to without, thin to thick walled, curved at apices to blunt. Linnaeus (1753) established the species *Trichosanthes anguina* and *T. cucumerina*. Haines (1924) recognized *T. cucumerina* L. var. *cucumerina* (L.) as a wild variant with short fruits and *T. cucumerina* var. *anguina* (L.) as a cultivated variant with elongated, snake-like fruits. Chakravarty (1982) treated *T. cucumerina* and *T. anguina* as two different species. Jeffrey (1980) recognized *T. anguina* as a variety of *T. cucumerina*. We herein also recognized on the basis of similar pattern of trichomes morphology *T. anguina* as a variety of *T. cucumerina*. Luffeae is a monogeneric tribe under subfamily Cucurbitoideae. The present study reveals similar pattern of trichomes morphology in *Trichosanthes* and *Luffa* species. A close relationship between Trichosantheae and Luffeae has also been suggested on the basis of molecular systematic study (Kocyan et al., 2007). The present study clearly reflects that the diversity of trichomes in Cucurbitaceae can be used in solving the taxonomic problem of narrowly delimited taxa as well as can be used as a character state in infrageneric classification. Accurate and rapid authentication of the medicinal cucurbits included in the present study (viz., *C. melo* var. *agrestis*, *D. palmatus*, *E. dargeelingensis*, *G. burmanicum*, *G. pentaphyllum*, *G. pubescens*, *H. diptrygia*, *L. echinata*, *M. heterophylla*, *M. leucocarpa*, *M. maderspatana*, *T. cordifolia*, *T. cucumerina*, *T. lepiniana* and *T. tricuspidata*) and their adulterants is difficult to achieve. Characteristics of trichomes micromorphology in cucurbits enumerated in the present study have potential for the authentication of medicinal cucurbits and their adulterants.

References

Ali, M.A., 2006. Taxonomic studies on the family Cucurbitaeae of eastern Bihar. Ph.D. Thesis. Tilka Manjhi Bhagalpur University, Bhagalpur, Bihar, India.

Ali, M.A., Pandey, A.K., 2006. Cucurbitaceae of Bihar: diversity and conservation. In: Trivedi, P.C. (Ed.), Global Biodiversity Status and Conservation. Pointer Publisher, Jaipur, Rajasthan, India, pp. 257–263.

Ali, M.A., Pandey, A.K., 2007. Cucurbitaceae of Eastern Bihar. PSRC Publication, India.

Ali, M.A., Pandey, A.K., Lee, J., 2009. Relationships among subfamily Cucurbitoideae (family Cucurbitaceae) from India inferred from ITS sequences of nrDNA. Phytomorphology 59 (3 and 4), 127–140.

Banerjee, A., Sinhababu, A., Kar, R.K., Mandal, S., 2004. Micromorphological studies of four fuel wood yielding tropical leguminous plant. Pak. J. Biol. Sci. 7 (1), 100–104.

Borril, M., 1961. Epidermal characteristic in the diploid subspecies of *Dactylis glomerata* L.. J. Linn. Soc. Bot. 56, 453–458.

Chakravarty, H.L., 1982. Fascicles of Flora of India Fascicle II Cucurbitaceae. Botanical Survey of India, Calcutta, India.

Edeoga, H.O., 1991. Comparative morphology of leaf epidermis in *Costus* after *C. lucanusianus* (Costaceae) complex and its systematic importance. Nig. Ann. Nat. Sci. 1, 25–30.

Edeoga, H.O., Ikem, C.I., 2001. Comparative morphology of the leaf epidermis in three species of *Boerhavia* L. (Nyctaginaceae). In: Maheshwari, J.K., Jain, A.P. (Eds.), Recent Research in Plant Anatomy and Morphology. Scientific Publishers, Jodhpur, India, pp. 197–205.

Hagerup, O., 1953. The morphology and systematics of leaves of Eriales. Phytomorphology 3, 459–464.

Haines, H.H., 1924. The Botany of Bihar and Orissa. Allard and Son and West Newman Ltd., London.

Haron, N.W., Moore, D.M., 1996. The taxonomic significance of leaf micromorphology in the genus *Eugenia* L. (Myrtaceae). Bot. J. Linn. Soc. 120 (3), 265–277.

Husain, S.Z., Aziz, K., Saleha, T.S., Jahan, N., 1994. Micromorphological studies of seven species of the genus *Medicago* L. (Fabaceae) from Pakistan. Pak. J. Bot. 26 (2), 409–419.

Jeffrey, C., 1980. A review of the Cucurbitaceae. Bot. J. Linn. Soc. 81, 233–247.

Jeffrey, C., 2005. A new system of Cucurbitaceae. Bot. Zhurn. 90, 332–335.

Kannabiran, B., Krishnamurthy, K.H., 1972. Morphology of foliar epidermis and taxonomy of the genus *Crotalaria*. Phytomorphology 24, 61–68.

Kocyan, A., Zhang, L.B., Schaefer, H., Renner, S.S., 2007. A multilocus chloroplast phylogeny for the Cucurbitaceae and its implications for character evolution and classification. Mol. Phylogenet. Evol. 44, 553–577.

Leelavathi, P.M., Ramayya, N., 1983. Structure, distribution and classification of plant trichomes in relation to taxonomy II. Caesalpinoideae. Ind. J. For. 6, 43–56.

Linnaeus, C., 1753. Species Plant. (1753) 2, 1008.

McWhorter, C.G., Ouzts, C., Paul, R.N., 1993. Micromorphology of johnsongrass (*Sorghum helepense*) leaves. Weed Sci. 41, 583–589.

Nyawuame, H.G.H., Gill, L.S., 1990. Epidermal morphology and histogenesis in some crop plants. Nig. J. Agron 4, 6–10.

Pandey, A.K., Varma, S.K., Ali, M.A., 2006. The genus *Luffa* in India: diversity and conservation. In: Trivedi, P.C. (Ed.), Global Biodiversity Status and Conservation. Pointer Publisher, Jaipur, Rajasthan, India, pp. 260–270.

Parveen, N.S., Murthy, K.S.R., Pullaiah, T., 2000. Leaf epidermal characters in *Crotalaria* sp. (Papilionoideae) from eastern Ghats. Phytomorphology 50, 205–212.

Pathan, A.K., Bond, J., Gaskin, R.E., 2008. Sample preparation for scanning electron microscopy of plant surfaces-Horses for courses. Micron 39, 1049–1061.

Rapisarda, A., Iauk, L., Ragusa, S., 1997. Micromorphological study on leaves of some *Cordia* (Boraginaceae) species used in traditional. Econ. Bot. 51 (4), 385–391.

Shanta, A., Radhakrishaniah, M., 2000. Cladistic analysis of selected genera of Cucurbitaceae. Phytomorphology 50 (1), 103–111.

Stace, C.A., 1984. The taxonomic importance of the leaf surface. In: Heywood, V.H., Moore, D.F. (Eds.), Current Concept of Plant Taxonomy. Academic Press, London, pp. 31–39.

Stebbins, G.W., Jain, S.K., 1960. A study of stomata development in *Allium* and *Commelina*. Dev. Biol. 1, 409–426.

Wang, Yu-Guo, Guang-Zhao, L.I., Zhang, Wen-Ju, You, J., Jia-Kuan, Chen, 2007. Leaf epidermal features of Rhododendron (Ericaceae) from China and their systematic significance. Acta Phytotax. Sin. 45 (1), 1–20.

Zhang, Li-Bing, Simmons, M.P., Kocyan, A., Renner, S.S., 2006. Phylogeny of the Cucurbitales based on DNA sequences of nine loci from three genomes: implications for morphological and sexual system evolution. Mol. Phylogenet. Evol. 39, 305–322.