Bangladesh J. Plant Taxon. **21**(1): 43-51, 2014 (June)

STOMATAL AND TRICHOME DIVERSITY IN *SENNA* MILL. FROM BANGLADESH

AYESA BEGUM, MD. OLIUR RAHMAN[1] AND MOMTAZ BEGUM

Department of Botany, University of Dhaka, Dhaka 1000, Bangladesh

Keywords: Senna; Stomata; Trichomes; Taxonomy; Bangladesh.

Abstract

Foliar epidermal characters of ten species of *Senna* Mill. found in Bangladesh are investigated. Anisocytic, anomocytic, paracytic, tetracytic and haxacytic stomata are found across the species. Anisocytic and paracytic stomata are common in all species. In addition to anisocytic and paracytic types, anomocytic stomata are found in *Senna sophera* and hexacytic stomata are observed in *S. auriculata*. Anticlinal wall is straight in *S. alata, S. hirsuta, S. occidentalis, S. siamea* and *S. tora*, curved in *S. auriculata, S. sophera* and *S. siamea*, and undulate in *S. hirsuta* and *S. occidentalis*. Glandular trichomes are observed in *S. hirsuta* and *S. occidentalis*, while non-gladular trichomes are predominant in the remaining species.

Introduction

Senna Mill. is a large, widespread genus and exhibits a high diversity of habits including herbs, shrubs, treelets, tall trees and lianas. The genus comprises 350 species and about 80% of its species occur in the American continent, while most of the remaining members are found in tropical Africa, Madagascar and Australia, and only a few species occur in South-eastern Asia and Pacific Island (Irwin and Barneby, 1982; Marazzi *et al*., 2006). Species of *Senna* were formerly included in *Cassia* L. *s.l.* (Irwin and Turner, 1960). Subsequent taxonomic treatments subdivided the *Cassia s.l.* into three distinct genera, *viz., Cassia s. str*., *Chamaecrista* Moench and *Senna* Mill. (Irwin and Barneby, 1981, 1982). *Senna* Mill. are characterized by presence of extrafloral nectaries, ebracteolate pedicels, straight or simply incurved filaments, flattened or cylindrical, irregularly dehiscent pods and areolate seeds, while in *Cassia* L., no extrafloral nectaries present, pedicels 2-bracteolate, filaments sigmoidally curved towards the base, pod indehiscent, and there is no areole on seed surface. *Chamaecrista* Moench is distinct by ciliolate anther-thecae along the suture, elastically dehiscent pod, smooth or pitted seed coat and absence of aerole on seed surface (Irwin and Barneby, 1982). The separation of *Senna* Mill. from *Cassia* L. was further established by taxonomic (Singh, 2001), phenetic (Boonkerd *et al*., 2005) and molecular studies (Acharya *et al*., 2011). In Bangladesh, *Senna* Mill. is represented by 11 species (Rahman *et al*., 2013).

Taxonomic relevance of vegetative anatomy in delimitation of taxa and establishment of itergeneric or interspecific relationships is well reported (Tomblinson, 1961; Stace, 1965; Kotresha and Seetharam, 2000; Tschan and Denk, 2012). Foliar anatomical features play an important role in distinguishing different groups of plants. Leaf is considered as the most varied organ anatomically in angiosperms which provides a variety of anatomical features that can be employed as useful taxonomic characters (Metcalfe and Chalk, 1950; Metcalfe, 1968; Stace, 1984). Foliar anatomical characters such as stomata and trichomes have been found instrumental in solving taxonomic problems. The taxonomic and phylogenetic significance of stomata and trichomes have long been recognized by various workers (Dilcher, 1974; Naik and Nirgude, 1981; Stace, 1984; Devi *et al*., 2013).

*Corresponding author. Email: prof.oliurrahman@gmail.com

Despite several studies based on foliar micromorphology have been made in defferent genera, *viz., Polygonum* (Lersten and Curtis, 1992), *Eugenia* (Fontenelle *et al.*, 1994), *Bauhinia* (Kotresha and Seetharam, 1995), *Hibiscus* (Shaheen *et al.*, 2009), *Fagopyrum* (Yasmin *et al*., 2010) and *Cynoglossum* (Akçin *et al*., 2012), the genus *Senna* Mill. received very little attention in this regard (Ogundipe *et al*., 2009). Therefore the present study was undertaken in order to explore the features of stomata and trichome in the genus *Senna* Mill. occurring in Bangladesh and to evaluate these characters for species delimitation and interspecific relationship.

Materials and Methods

Ten species of *Senna* Mill. used in this study are *Senna alata* (L.) Roxb., *S. auriculata* (L.) Roxb., *S. hirsuta* (L.) Irwin & Barneby, *S. obtusifolia* (L.) Irwin & Barneby, *S. occidentalis* (L.) Link, *S. siamea* (Lam.) Irwin & Barneby, *S. sophera* (L.) Roxb., *S. surattensis* (Burm. f.) Irwin & Barneby, *S. timoriensis* (DC.) Irwin & Barneby and *S. tora* (L.) Roxb. The localities of these species along with the voucher numbers are appended in Table 1. Plant materials collected from different parts of the country as well as herbarium specimens deposited in Dhaka University Salar Khan Herbarium (DUSH) and Bangladesh National Herbarium (DACB) were studied.

Leaf samples were cut into small square pieces and soaked in a petridish containing KOH solution for 7-8 days. Then they were washed with tap water followed by treatment with hydrogen peroxide for 3-4 days till clearing of leaves. After that they were treated with 50% alcohol for 2 hours and then with safranin for 16-18 hours. The leaf tissues were treated again with 70% alcohol for 2 hours. Finally, the samples were treated with a mixture of alcohol and xylol in a 3:1 ratio and mounted in 70% glycerin. The samples were prepared in clean, dry and oil free slides and observed under compound microscope. Photomicrograph of each species was taken using Nikon Eclipse 200 camera in addition to the line drawing of stomata.

Table 1. List of species of *Senna* Mill. along with voucher specimens used in the present study.

No.	Species	Voucher specimens
1	*Senna alata* (L.) Roxb.	Dhaka: Dhaka University campus, 23.12.2011, Ayesa 65 (DUSH)
2	*S. auriculata* (L.) Roxb.	Dhaka: Sher-e-Bangla Agricultural University compound, 26.1.2011, Ayesa 07 (DUSH)
3	*S. hirsuta* (L.) Irwin & Barneby	Gazipur: Gazipur, 30.6.2011, Ayesa 40 (DACB)
4	*S. obtusifolia* (L.) Irwin & Barneby	Cox's Bazar: Teknaf, Mouchuni, 24.4.2011, Ayesa 32 (DUSH)
5	*S. occidentalis* (L.) Link	Dhaka: Dhaka University campus, 26.12.2010, Ayesa 02 (DUSH)
6	*S. siamea* (Lam.) Irwin & Barneby	Dhaka: Tejgaon, Old Airport, 27.12.2011, Ayesa 74 (DUSH)
7	*S. sophera* (L.) Roxb.	Dhaka: Dhaka University Campus, 30.4.11, Aeysa 33 (DUSH)
8	*S. surattensis* (Burm. f.) Irwin & Barneby	Dhaka: Dhaka University campus, 20.12.2011, Ayesa 47 (DUSH)
9	*S. timoriensis* (DC.) Irwin & Barneby	Chittagong Hill Tracts: Ruma P.S., Changnakra, 25.1.1965, M. S. Khan 1166 (DACB).
10	*S. tora* (L.) Roxb.	Dhaka: Dhaka University Botanical garden, 26.12.2011, Ayesa 69 (DUSH)

Results and Discussion

The genus *Senna* offers different types of stomata and trichomes. The shape of epidermal cells, nature of anticlinal walls, types of stomata and number of stomata per microscopic field are presented in Table 2. The epidermal cells are irregular or polygonal in outline. Polygonal cells are observed in *S. obtusifolia, S. siamea* and *S. tora*, while irregular types of cells are evident in *S. sophera*. The remaining species bear both irregular and polygonal cells (Table 2). The anticlinal walls are straight, undulate and curved across the genus. Straight anclinal walls are found in *S. obtusifolia, S. siamea* and *S. tora*; anticlinal walls are curved in *S. auriculata, S. sophera* and *S. surattensis,* and they are undulate, curved and straight in *S. hirsuta* and *S. occidentalis*. Stomata are mostly paracytic and anisocytic, however, tetracytic, anomocytic and hexacytic stomata have also been documented. Paracytic and anisocytic stomata are observed in all species, while anomocytic stomata are found only in *S. sophera*, and hexacytic stomata only in *S. auriculata* along with other types. Paired anisocytic and paired tetracytic stomata are present only in *S. auriculata.* Paired paracytic stomata are observed in *S. sophera* and *S. hirsuta* (Table 2; Figs 1&2). Amphistomatic stomata have been observed in all species employed, while hypostomatic stomata is found only in *S. siamea*.

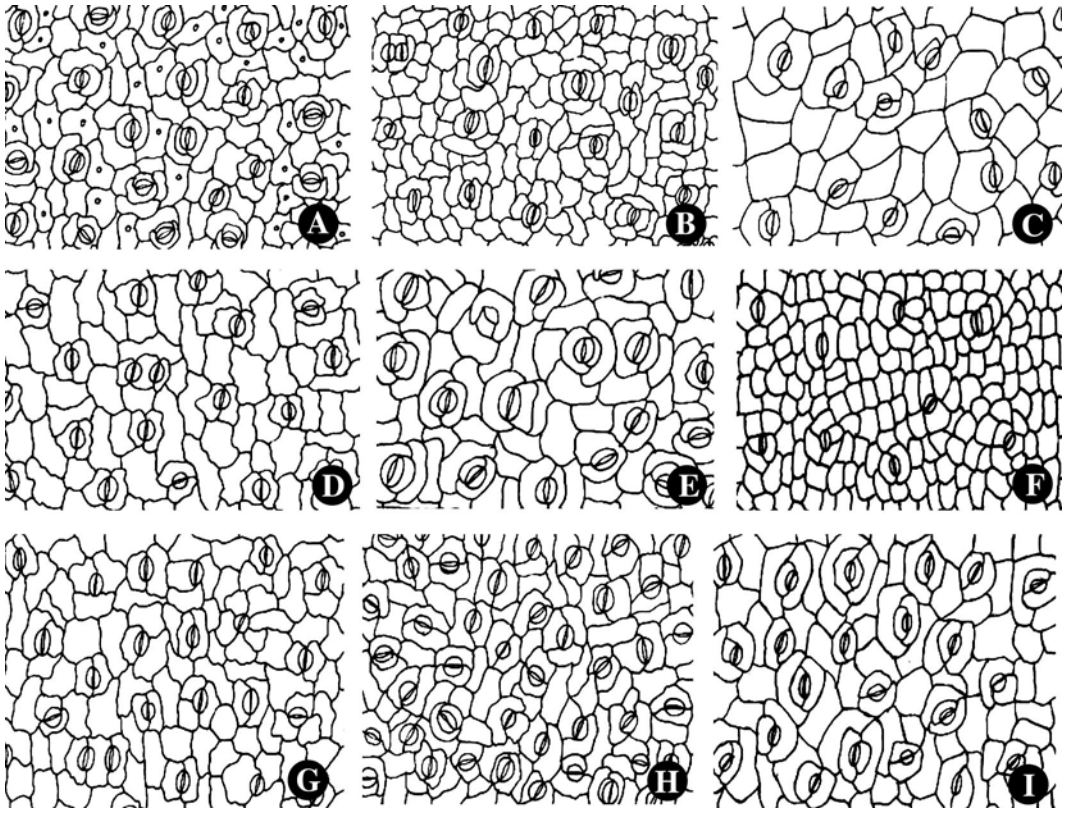

Fig. 1. Line drawings of different types of stomata found in *Senna* Mill., A) *Senna alata*, B) *S. auriculata*, C) *S. hirsuta*, D) *S. obtusifolia*, E) *S. occidentalis*, F) *S. siamea,* G) *S. sophera*, H) *S. surattensis*, I) *S.tora.*

Table 2. Foliar stomatal variation in *Senna* Mill. species used in the present study.

Species	Epidermal cell		Anticlinal wall		Types of stomata		Number of stomata/ microscopic field	
	Adaxial	Abaxial	Adaxial	Abaxial	Adaxial	Abaxial	Adaxial	Abaxial
Senna alata	Polygonal	Irregular	Straight	Straight, Curved	Paracytic, Anisocytic	Paracytic	29	50
S. auriculata	Polygonal, Irregular	Polygonal, Irregular	Curved	Curved	Paracytic, Anisocytic, Paired tetracytic	Paracytic, Anisocytic, Tetracytic, Hexacytic, Paired anisocytic	44	39
S. hirsuta	Polygonal, Irregular	Polygonal, Irregular	Straight, Undulate	Straight, Curved	Paracytic	Paracytic, Anisocytic, Paired paracytic	35	39
S. obtusifolia	Polygonal	Polygonal	Straight	Straight	Paracytic	Paracytic, Anisocytic	32	37
S. occidentalis	Polygonal, Irregular	Polygonal, Irregular	Straight, Undulate	Straight, Curved	Paracytic	Paracytic, Anisocytic, Tetracytic	21	46
S. siamea	Polygonal	Polygonal	Straight	Straight	-	Paracytic, Anisocytic, Tetracytic	-	0-7
S. sophera	Irregular	Irregular	Curved	Curved	Paracytic, Anisocytic, Anomocytic, Tetracytic, Paired paracytic	Paracytic, Anisocytic, Tetracytic	28	58
S. surattensis	Polygonal, Irregular	Polygonal, Irregular	Curved	Curved	Paracytic, Anisocytic, Tetracytic	Paracytic, Anisocytic	54	51
S. tora	Polygonal	Polygonal	Straight	Straight	Paracytic, Anisocytic	Paracytic, Anisocytic	32	25

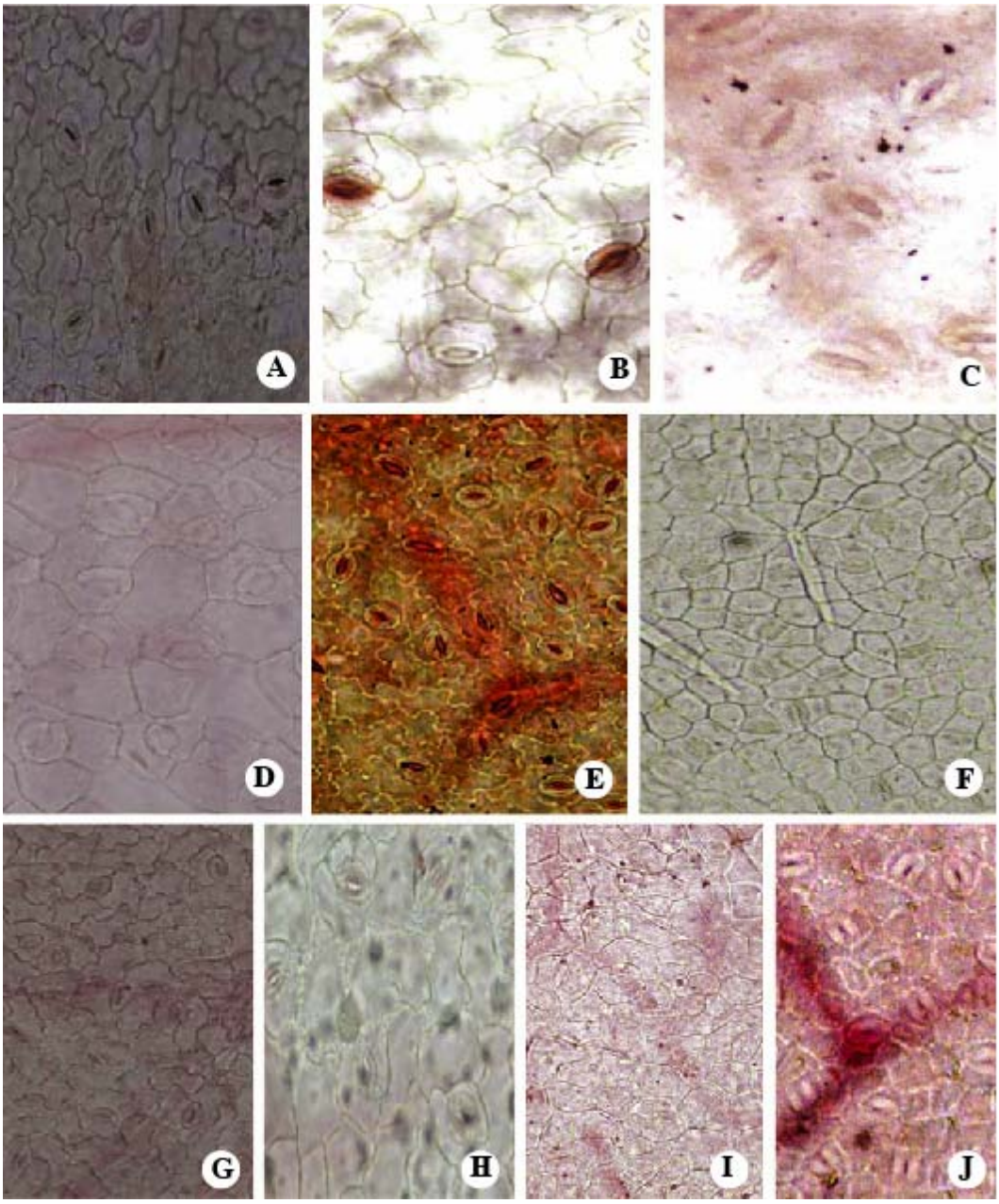

Fig. 2. Different types of stomata in *Senna* Mill. A) *Senna alata*, B) *S. auriculata*, C) *S. hirsuta*, D) *S. obtusifolia*, E) *S. occidentalis*, F) *S. siamea*, G) *S. sophera*, H) *S. surattensis*, I) *S. tora*, J) *S. timoriensis*.

The study reveals that both glandular and non-glandular trichomes are found in *Senna*. The trichomes observed in *Senna* vary in structure, form and distribution. Glandular multicellular trichomes are evident only in the abaxial surface of *S. hirsuta* and *S. occidentalis*. The number of

cells varies from 5 to 7. However, no glandular unicellular trichomes are found in any species employed in the present study (Table 3). Non-glandular trichomes are evident in all species except *S. occidentalis*. Non-glandular multicellular trichomes are distinct in *S. hirsuta*, *S. obtusifolia* and *S. tora*, whereas, non-glandular unicellular trichomes are observed in eight species. In *S. alata, S. hirsuta* and *S. siamea* unicellular trichomes are sparsely noticed. Non-glandular unicellular trichomes may be conical or papilose. Very rarely uncinate trichomes are found only in *S. alata*. Non-glandular multicellular trichomes are consisted of 2-8 cells, which also vary greatly in size, shape and number of cells.Verrucose type of trichome wall are documented in *S. tora, S. obtusifolia, S. siamea, S. surattensis* and smooth walled trichomes are seen in *S. hirsuta, S. auriculata, S. timoriensis* (Fig. 3). The longest type of multicellular trichomes are present with up to 8 cells, comprising two or more basal cells ended by a much elongated apical cell in *S. hirsuta*. The pedastal cells might vary from 4 to 7. Multicellular verrucose trichome present in *S. tora* and *S. obtusifolia.* The cells of the stalk are almost equal in length. Smooth walled non-glandular multicellular trichomes are present in *S. hirsuta*, consisting of 2-6 cells and the cells may be equal or unequal in size. The upper cells always larger than the basal ones and become narrower towards the apex. *S. occidentalis* and *S. hirsuta* can easily be distinguished from the other species of *Senna* by presence of glandular multicellular trichomes.

Table 3. Trichome variation in *Senna* Mill. species employed in the present study.

Species	Glandular trichome				Non-glandular trichome			
	Unicellular		Multicellular		Unicellular		Multicellular	
	Adaxial surface	Abaxial surface	Adaxial surface	Abaxial surface	Adaxial surface	Abaxial surface	Adaxial surface	Abaxial surface
Senna alata	–	–	–	–	+ (very few)	+ (very few)	–	_
S. auriculata	–	–	–	–	++	++	_	–
S. hirsuta	–	–	–	+ (very few)	–	+ (very few)	+++ (2-6 celled)	+++ (2-4 celled)
S. obtusifolia	–	–	–	–	++	–	–	++ (1-7 celled)
S. occidentalis	–	–	–	++ (5-7 celled)	–	–	–	–
S. siamea	–	–	–	–	+ (very few)	+ (very few)	–	–
S. sophera	–	–	–	–	++	++	–	–
S. surattensis	–	–	–	–	++	++	–	–
S. timoriensis	–	–	–	–	++	+++	–	–
S. tora	–	–	–	–	–	–	–	++ (2-4 celled)

– = absent, + = sparsely present, ++ = moderately present, +++ = densely present.

The taxonomic value of leaf epidermal characters have been received much attention in the recent past, even the taxonomic monographs are now considered incomplete without micromorphology of the epidermis (Rejdali, 1991). In this context, however, little is known in the genus *Senna*. Recently Ogundipe *et al*. (2009) and Shaheed and Illoh (2010) studied foliar micromorphology of six species of *Senna* from Nigeria. The present study bridges the gaps in our knowledge of the some additional species of the genus *Senna* after Ogundipe *et al.* (2009) and Shaheed and Illoh (2010).

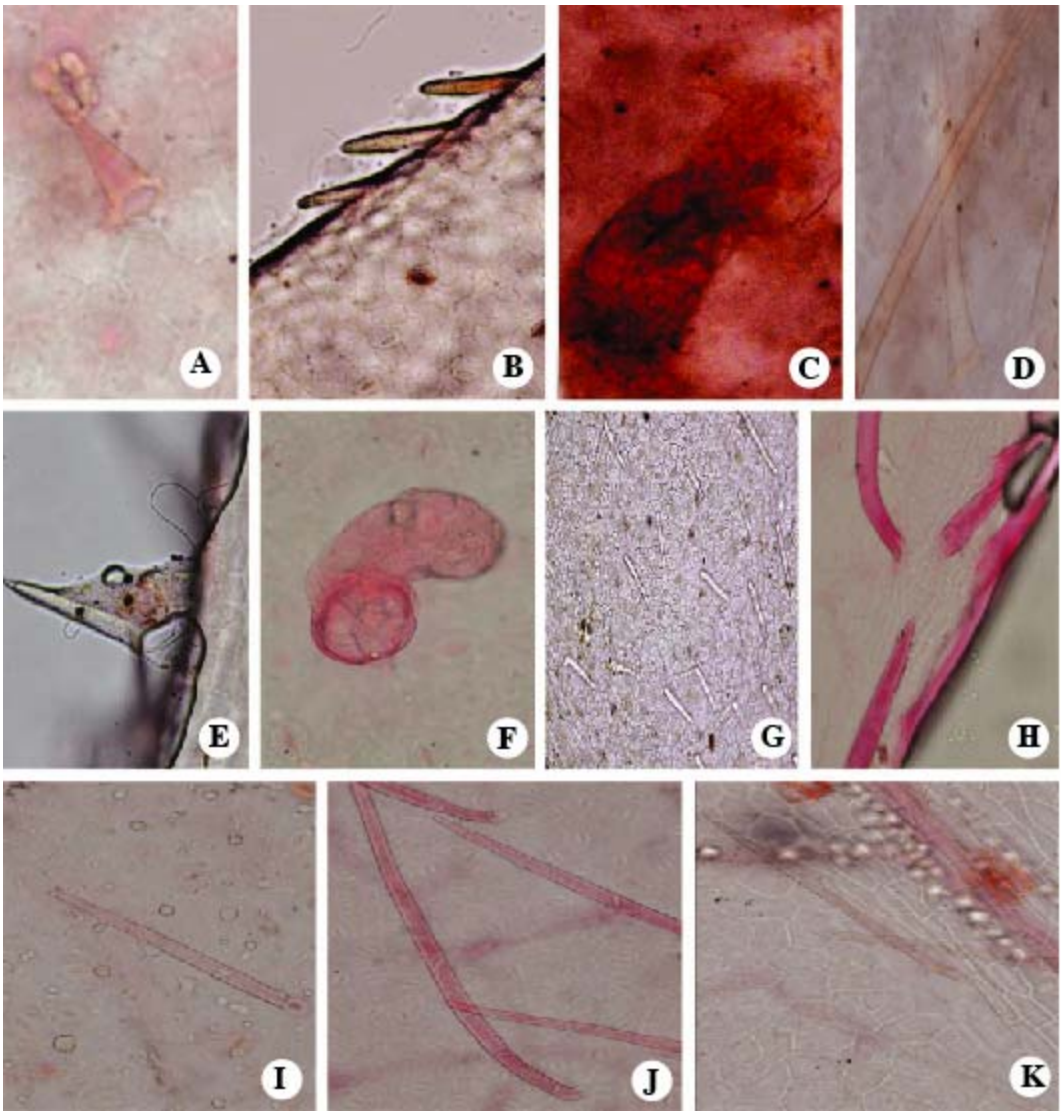

Fig. 3. Trichome diversity in *Senna* Mill., A) *Senna alata*, B) *S. auriculata*, C,D) *S. hirsuta*, E) *S. obtusifolia*, F) *S. occidentalis*, G) *S. siamea*, H) *S. sophera*, I) *S. surattensis*, J) *S. tora*., K) *S. timoriensis*.

The present study reveals that the foliar epidermal characters are important in taxonomic identification and species relationships in the members of the *Senna* examined. In our study *S. obtusifolia* and *S. tora* have been found closely related as evidenced by their epidermal cells which are polygonal in shape, and anticlinal walls are straight both in *S. obtusifolia* and *S. tora*. Moreover, paracytic and anisocytic stomata are common in these two species indicating a close relationships among them. In a morphometric study of the genus *Senna* Rahman *et al*. (2013) showed that *S. obtusifolia* and *S. tora* are very closely related as attested by the following shared characters: leaflets obovate, stipules linear, falcate, inflorescence short-racemose, axillary, ovary ribbed, style glabrous, stigma truncate and pod linear or subtetragonous, which has been found

consitent with our study. Morphologically *S. alata* and *S. auriculata* are closely allied, however, the epidermal features do not support a close association between them. *S. auriculata* is characterized by presence of hexacytic stomata, while *S. sophera* is distinct by presence of anomocytic stomata. Saheed and Illoh (2010) observed that in *S. alata*, the adaxial epidermal cells are polygonal or irregular, while the abaxial cells are irregular in shape, and this species exhibited non-glandular trichomes. Our study presenting similar types of epidermal cells and non-glandular trichomes in *S. alata* support the work of Saheed and Illoh (2010). *S. obtusifolia* is characterized by presence of polygonal epidermal cells, straight anticlinal walls, and paracytic and anisocytic stomata, however, no anomocytic stomata were found in this species as documented by Saheed and Illoh (2010).

The study also unveils a close relationship between *S. hirsuta* and *S. occidentalis* as they share the following common characteristics: both polygonal and irregular epidermal cells, straight and undulate anticlinal walls in the adaxial surface, straight and curved walls in the abaxial surface, paracytic stomata in the adaxial surface, and both paracytic and anisocytic stomata in the abaxial surface (Table 2). *S. hirsuta* and *S. occidentalis* can also be differentiated from the remaining species by presence of multicellular glandular trichomes as observed in the abaxial surface (Table 3). While studying on the Nigerian *Senna* species Ogundipe *et al*. (2009) showed that *S. hirsuta* and *S. occidentalis* are closely allied as they bear paracytic and anisocytic types of stomata, and the epidermal cells are polygonal and irregular in both species. Results obtained from the present study have been found congruent with that of Ogundipe *et al*. (2009). The close affinity between *S. hirsuta* and *S. occidentalis* is also evidenced by cytological investigation where the somatic chromosome number 2n=28 was reported for these species (Bir and Kumari, 1980).

Acknowledgement

We would like to thank Prof. Dr. Md. Abul Hassan, Department of Botany, University of Dhaka for his cooperartion during the course of the study. Thanks are also due to the authority of Bangladesh National Herbarium for allowing us to examine the herbarium materials.

References

Acharya, L., Mukherjee, A.K. and Panda, P.C. 2011. Separation of the genera in the subtribe *Cassiinae* (Leguminosae: Caesalpinioidae) using molecular markers. Acta Botanica Brasilica **25**(1): 223-233.

Akçin, O.E., Çoşkunçelebi, K. and Şenel, G. 2012. Foliar anatomy of *Cynoglossum* L. (Boraginaceae) from north Anatolia, Turkey. Bangladesh J. Plant Taxon. **19**(2): 101-108.

Bir, S.S. and Kumari, S. 1980. Cytological evolution of the Leguminous flora of the Punjub plain. *In*: Bir, S.S. (Ed.), Recent Researches in Plant Science. Kalyani Publishers, Ludhiana, India, pp. 261-271.

Boonkerd, T., Pechsri, S. and Baum, B.R. 2005. A phenetic study of *Cassia s.l.* (Leguminosae-Caesalpinioideae: Cassieae: Cassiinae) in Thailand. Plant Syst. Evol. **252**: 153-165.

Devi, N.J., Padma, Y., Narasimhudu, C.L. and Raju, R.R.V. 2013. Diversity of stomata and trichomes in *Euphorbia* L. – I. Bangladesh J. Plant Taxon. **20**(1): 27-38.

Dilcher, D.L. 1974. Approaches to the identification of angiosperm leaf remains. Bot. Rev. **40**: 1-157.

Fontenelle, G.B., Costa, C.G. and Machado. R.D. 1994. Foliar anatomy and micromorphology of eleven species of *Eugenia* L. (Myrtaceae). Bot. J. Linn. Soc. **116**: 111-133.

Irwin, H.S. and Turner, B.L. 1960. Chromosomal relationships and taxonomic considerations in the genus *Cassia*. Am. J. Bot. **47**: 309-318.

Irwin, H.S. and Barneby, R.C. 1981. Tribe 2. *Cassiae* Bronn (1822). *In*: Pohlhill, R.M. and Raven, P.H. (Eds), Advances in Legume Systematics, Part 1. Royal Botanic Gardens, Kew, UK, pp. 97-106.

Irwin, H.S. and Barneby, R.C. 1982. The American Cassiinae. Memoir. New York Bot. Gard. **35**: 1-918.

Kotresha, K. and Seetharam, Y.N. 1995. Epidermal studies in some species of *Bauhinia* L. (Caesalpinioideae). Phytomorphology **45**(1&2): 127-137.

Kotresha, K. and Seetharam, Y.N. 2000. Epidermal micromorphology of some *Cassia* L. (Caesalpiniaceae). Phytomorphology **50**(3&4): 229-237.

Lersten, N.R. and Curtis, J.D. 1992. Foliar anatomy of *Polygonum* (Polygonaceae)*:* Survey of epidermal and selected internal structures. Plant Syst. Evol. **182**(1-2): 71-106.

Marazzi, B., Endress, K.P., de Queiroz, L.P.and Conti, E. 2006. Phylogenetic relationships within *Senna* (Leguminosae, Cassiinae) based on three chloroplast DNA regions: Patterns in the evolution of floral symmetry and extrafloral nectaries. Am. J. Bot. **93**(2): 288-303.

Metcalfe, C.R. and Chalk, L. 1950. Anatomy of the Dicotyledons, Vol. **2**. Oxford, pp. 1014-1024.

Metcalfe, C.R. 1968. Current development in systematic plant anatomy. *In*: Heywood, V.H. (Ed.), Modern Methods in Plant Taxonomy. Academic Press, London, New York, pp. 45-57.

Metcalfe, C.R. and Chalk, L. 1979. Anatomy of the Dicotyledons, Second edition, Vol. **1.** Clarendon Press, pp. 63-75.

Naik, V.N. and Nirgude, S.M. 1981. Anatomy in relation to taxonomy of *Chlorophytum* (Liliaceae). Indian J. Bot. **4**(2): 48-60.

Ogundipe, O.T., Kadiri, A.B. and Adekanmbi, O.H. 2009. Foliar epidermal morphology of some Nigerian species of *Senna* (Caesalpiniaceae). Indian J. Sci. & Tech. **2**(10): 5-9.

Rahman, M.O., Rahman, M.Z. and Begum, A. 2013. Numerical taxonomy of the genus *Senna* Mill. from Bangladesh. Bangladesh J. Plant Taxon. **20**(1): 77-83.

Rejdali, M. 1991. Leaf micromorphology and taxonomy of North African species of *Sideritis* L. (Lamiaceae). Bot. J. Linn. Soc. **107**: 67-77.

Saheed, S.A. and Illoh, H.C. 2010. A taxonomic study of some species in *Cassiinae* (Leguminosae) using leaf epidermal characters. Not. Bot. Hort. Agrobot. Cluj **38**(1): 21-27.

Shaheen, N., Ajab, M., Hayat, M.Q. and Yasmin, G. 2009. Diversity of foliar trichomes and their systematic relevance in the genus *Hibiscus* (Malvaceae). Int. J. Agric. Biol. **11**: 279-284.

Singh, V. 2001. Monograph on Indian subtribe *Cassiinae* (Caesalpiniaceae). Scientific Editions, Jodhpur, India.

Stace, C.A. 1965. Cuticular studies as an aid to plant taxonomy. Bull. Br. Mus. Nat. Hist. **4**: 1-78.

Stace, C.A. 1984. The taxonomic importance of the leaf surface. *In:* Heywood, V.H. and Moore, D.M. (Eds), Current Concepts in Plant Taxonomy. Academic Press, London, pp. 67-94.

Tomblinson, P.B. 1961. Anatomical approach to the classification of the Musaceae. Bot. J. Linn. Soc. **55**: 779-809.

Tschan, G.F. and Denk, T. 2012. Trichome types, foliar indumentum and epicuticular wax in the Mediterranean gall oaks, *Quercus* subsection Galliferae (Fagaceae): implications for taxonomy, ecology and evolution. Bot. J. Linn. Soc. **169**: 611-644.

Yasmin, G., Khan, M.A., Shaheen, N. and Hayat, M.Q. 2010. Micromorphological investigation of foliar anatomy of *Fagopyrum* Mill. and *Rumex* L. of Polygonaceae. Pak. J. Bot. **42(**1): 47-57.

(Manuscript recieved on 12 May 2013; revised on 29 May 2014)