Adaptation and morphotaxonomical implications of reproductive structures in some Indian Cassiinae

S. K. Mishra[1]*, D. K. Chauhan[2], S. K. Singh[3] and G. K. Srivastava[2]

[1]Department of Botany, Ewing Christian College, Allahabad-211003, India
[2]Department of Botany, University of Allahabad, Allahabad-211002, India
[3]Birbal Sahni Institute of Palaeobotany, 53 University Road, Luchnow-226007, India
E-mail: sanjaiy_1975@yahoo.co.in; dkchauhanau@yahoo.com;
sanjai_sk2002@yahoo.co.in; srivastavagkau@gmail.com
*Corresponding author

ABSTRACT

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Micro-morphological features of androecium and gynoecium, which have been less considered for taxonomic purposes, show considerable variations in genera *Cassia* Linn. and *Senna* Mill. belonging to sub-tribe Cassiinae of family Caesalpiniaceae. The androecial complement, consisting of 10 stamens with polleniferous or sterile anthers, show morphological as well as functional heteranthery. Variations have been recorded in shape, size, structure and mode of dehiscence of anthers. Spectacular variations exist between and among the species of *Cassia* and *Senna* in micro-morphology of stigma especially in shape, size of stigmatic orifice and trichomes fringing it. Poricidal anther dehiscence, enantiostylous carpel with minute internal stigma and stigmatic orifice fringing with variable trichomes, specifically evolved as an adaptation to promote specific pollination strategy mediated by insects, bear infrageneric taxonomic implications. The micro-morphological features of pistil, unique to each species, helps in segregation and identification of closely related species of genus *Cassia* and *Senna*, as well as in reviewing the taxonomic status of sub-tribe Cassiinae. These micro-morphological structures provide substantive evidence in resolving taxonomic riddles between closely related species of *Cassia* (*C. nodosa, C. javanica, C. renigera* and *C. roxburghii*) and *Senna* (*S. obtusifolia* and *S. tora*; *S. sulfurea* and *S. surattensis*).

Key-words: Cassiinae, *Cassia*, *Senna*, heteranthery, enantiostyly, stigmatic orifice, eastern Uttar Pradesh, India.

INTRODUCTION

Sub-tribe Cassiinae (formerly genus *Cassia* L.), belonging to tribe Cassieae of family Caesalpiniaceae, was established by raising the genus *Cassia* L. sensu lato to the level of sub-tribe and elevating the previous three sub-genera to the generic rank (Irwin & Barneby 1982). It includes three morphologically distinct genera, viz. *Cassia* L. sensu str., *Senna* Mill. and *Chamaecrista* Moench., comprising about 600 species of herbs, shrubs and trees, distributed in tropics and subtropics (Irwin & Turner 1960). Singh (2001) described 62 taxa of sub-tribe Cassiinae belonging to genera *Cassia* (8 species), *Chamaecrista* (11 species) and *Senna* (43 species) from Indian region. Cassiinae shows considerable variation in floral morphology, particularly that of androecium and gynoecium. Floral features of different species under sub-tribe have been studied by Knuth (1904, 1906), Van der Pijl (1954), Faegri

and Van der Pijl (1966), Procter and Yeo (1973), Dulberger (1981), Pardha Sardhi and Mohan Ram (1981), Müller H. (1881, 1882), Müller F. (1883), Wolfe and Estes (1991), Chauhan et al. (2003), Laporta (2005) and Anuradha (2009). Venkatesh (1956a, b, 1957) made a comprehensive study on heteranthery and mode of dehiscence of anthers in species of all the three genera of subtribe Cassiinae. Stigma morphology of some species under Cassiinae has been studied by Owens (1985, 1989), Owens and Lewis (1989) and Dulberger et al. (1994). They reported significant variation in pore size of stigmatic orifice and structure of fringing trichomes on stigma. The dimorphism in floral morphology in different species under Cassiinae has been considered as device for promoting cross pollination (Todd 1882, Knuth 1906) or for promoting pollination between left and right handed morphs, increasing geitonogamy (Bahadur et al. 1990). Thorpe and Estes (1975) interpreted enantiostyly as enigma. Sub-tribe Cassiinae shows specifically evolved floral features that promote buzz pollination (Buchmann & Hurley 1978, Buchmann 1983, Gottsberger & Gottsberger 1988, Laporta 2005). Heslop-Harrison (1981) recognized the role of stigma characteristics in angiosperm systematics but the micro-morphological structures of androecium and gynoecium have been least considered for taxonomic purposes and in inferring their role in reproductive success of species under Cassiinae. Therefore present study aims to provide additional information, based on light and scanning electron microscopic observation, on micro-morphology of anthers and carpel, its possible implications in taxonomy and their adaptive significance in relation to specific strategy evolved to promote effective and economic pollination in different taxa under Cassiinae.

MATERIAL AND METHODS

The present investigation on floral morphology was carried out on five species of the genus Cassia, viz. C. fistula L., C. javanica L., C. nodosa Buch.-Ham. ex Roxb, C. renigera Wall. ex Benth, C. roxburghii DC. and seven species of the genus Senna, viz. S. alata (L.) Roxb., S. obtusifolia (L.) Irwin & Barneby, S. occidentalis (L.) Roxb., S. siamea (Lam.) Irwin & Barneby, S. sulfurea (DC. ex Collad.) Irwin & Barneby., S. surattensis Burm. and S. tora L., growing in different parts of eastern Uttar Pradesh for three consecutive flowering seasons (2009-2011). The voucher specimens of all the presently investigated species are deposited in the Duthie Herbarium, Department of Botany, University of Allahabad, India. Transparency slides of fixed stamens and carpels were made for micro-morphological observation. For SEM studies, the carpels were thoroughly washed and dehydrated with absolute alcohol to avoid alteration in micro-morphological features. The dehydrated specimens were stuck to the double-sided scotch tape. The specimens were mounted on the aluminium stubs and subjected to sputter coating by employing gold palladium (Au/Pd) target. The coated specimens were stereo-scanned under suitable magnification at an accelerating potential of 15 KV Leo with 432 setero-scanning electron microscope at Birbal Sahni Institute of Palaeobotany, Lucknow, India.

FLORAL DESCRIPTION

Characters of stamens and carpel and their variations are summarized for each species in Tables 1 and 2. Light micrographs and SEM micrographs, illustrating the variations, are presented in Plates 2-4. The flowers in all the species of the genera Cassia and Senna are bisexual. Flower forms vary considerably, however they are regarded as key character in the classification of genera. In Cassia, the flowers are zygomorphic but in Senna, they vary from zygomorphic to highly asymmetrical forms due to perianth and displaced pistils. In Cassiinae, inflorescence is usually racemose type but variations have been observed in different species. The colour of the flower may be yellow (C. fistula, S. alata, S. siamea S. obtusifolia, S. tora, S. occidentalis, S. sulfurea and S. surattensis) or red and pink (C. javanica, C. nodosa, C. renigera and C. roxburghii). They are arranged in axillary or

terminal racemes, corymbs or fasicles. In *S. alata*, yellow flowers are arranged in axillary racemes that are condensed and form waxy yellow spikes that resemble fat candles before the individual blossom opens. Flowers are mostly bracteate, but bracteoles are absent in *Senna* species however they are present in *C. fistula, C. javanica, C. nodosa, C. renigera* and *C. roxburghii*. The bract structure varies in different species. They may be persistent or caducous, pubescent or glabrous. Calyx consists of five sepals which are mostly free (*C. fistula*) or the two upper most connate or all fused. The aestivation may be imbricate or quincuncial (*S. tora, S. obtusifolia*), odd sepal is anterior, sepals are obtuse, nearly equal (*S. alata, S. surattensis, S. sulfurea, S. occidentalis* and *S.*

Table 1. Characteristics of stamens in various species of *Senna* and *Cassia*.

Taxa	Characteristics
Senna alata (L.) Roxb.	Fertile stamens 7 of which 2 anterior are longer swollen, falcate and tailed at base, rest 5 stamens are straight, all anthers dehiscing by apical pores and through longitudinal rim, staminodes 3.
Senna obtusifolia (L.) Irwin & Barneby	Fertile stamens 7, 3 abaxial longer stamens up to 1 cm long with 5 mm long anthers which are narrowed at the apex like neck of the bottle (Plate 2, figure D). 4 lateral stamens are medium sized up to 7 mm length with 4 mm long anthers rounded at the top (Plate 2, figure C), filaments are hairy and anther dehisces by apical pores, 3 adaxial stamens are sterile and they are reduced to wedge shaped staminodes (Plate 1, figure G).
Senna tora L.	10 stamens arranged in two whorls. Fertile stamens 7, three of abaxial sides are larger, 6-10 mm long with anther length of about 5 mm with creamy slightly curved anther lobes; 4 lateral stamens are equal medium sized with 6-7 mm length and 3-4 mm long anthers with straight creamy anther lobes. All anthers are rounded at the apex dehiscing by terminal pores (Plate 2, figure B); 3 adaxial stamens two of the inner whorls and one of the outer whorls are reduced to wedge shaped staminodes.
Senna occidentalis (L.) Roxb.	Fertile stamens 6, unequal, 2 anterior abaxial stamens are 6-9 mm long, falcate, filament flat, deep yellow, anther 5 mm long brown. Out of four lateral stamens, 2 lateral adaxial stamens are 4-6 mm long, straight with winged filament and 2 lateral abaxial stamens are smaller, 2-3 mm long, straight; 3 adaxial and 1 abaxial stamens are reduced to linear oblong spatulate staminode. Anther pores sub-apical with hood (Plate 1, figure A, Plate 2, figures A, F).
Senna siamea (Lam.) Irwin & Barneby	There are ten subequal stamens in two whorls of 5 each. Out of five stamens of outer whorl, two lateral abaxial stamens are large, up to 1.5 cm in length with 5 mm long anther with bent tip. Three adaxial small stamens (two outer and one inner whorl) are reduced to staminodes, all anthers are yellow ellipsoid, basifixed, sagittate. The large and medium sized anthers dehisce by subterminal pores, while three small adaxial anthers fail to dehisce and are devoid of pollen grains or may contain pollen grains.
Senna sulfurea (DC. ex Collad) Irwin & Barneby	10 stamens, all fertile, subequal; anthers with narrow and deflexed top, lanceolate, dehiscing by apico-lateral slits.
Senna surattensis Burm.	10 stamens, subequal, all fertile; anthers with narrow and deflexed top (Plate 1, figure F), dehiscing by apical pores and lateral rims.
Cassia fistula L.	10 stamens occurring in two whorls are of three sizes, 3 long 4 medium and 3 short. Of the five stamens belonging to the outer whorl, three abaxial stamens are longest,. The anthers of the outer whorl are ellipsoidal or obovate-oblong and flattened with blunt ends showing incomplete longitudinal dehiscence at their tips by means of short slits. Four of the stamens of the inner whorl are medium sized and fifth is short and erect. The anthers of this whorl are obovate pear shaped with sub basal pores through which pollen are dispersed. Anthers borne by the short stamens in both the whorls are small indehiscent with or without pollen.
Cassia javanica L.	10 bright yellow stamens, all fertile, arranged in two whorls; 3 lower stamens are long and prominent and project in a double curve, swell out markedly in the middle (Plate 1, figure C) and then bend inwards, they are crowned with large brown ovate anthers, anther dehiscing by lateral slits (Plate 2, figure G). 7 stamens are smaller 6-12 mm long, straight, without swelling crowned with yellow anthers.
Cassia nodosa Buch-Ham ex Roxb.	10 stamens, unequal, all fertile. Three lower stamens are longer and prominent, they project in a double curve. Each filament is 2-2.5 cm long and characterized by the presence of a distinctive globular swelling in the middle (Plate 1, figure B), these nodes like swelling in the middle of the stamens give the tree its name. 7 stamens are half as long, almost straight, anthers are ovate and brown in colour, anther dehiscing by two basal pores and through longitudinal rim (Plate 2, figure H).
Cassia renigera Wall. ex Benth.	10 stamens all fertile, three lower stamens are longer with double bend in the filament, filaments are swollen in the centre and much curled and crowned with tender green anther. There are 4 smaller median stamens and 3 quite small erect ones. All of these are capped with anthers.
Cassia roxburghii DC	10 stamens, 3 long, 4 medium and 3 short, of the five stamens belonging to the outer whorl three are long, one deflected towards style, other two lie opposite, their filaments are twice curved, 2.5-3 cm long without globular swelling (Plate 1, figure A), anteriorly placed, glabrous. The anther lobes are incurved, ellipsoid and pink, two stamens are short and posteriorly placed. Four of the stamens of the inner whorl are medium sized and fifth is short. The long anther dehisces by subterminal slits (Plate 2, figure E) while the medium anther dehisces by sub-basal pores. Three small adaxial stamens have small anther lobes without pollen grains, i.e. they are reduced to staminode.

Table 2. Characteristics of carpel in various species of *Senna* and *Cassia*.

Taxa	Characteristics
Senna alata (L) Roxb.	Carpel surface shows densely dispersed spiny erect trichomes towards lower portion, while the upper portion is glabrous or occasionally shows dispersed trichomes. Style deflexed towards left or right side, stigma with circular orifice surrounded by 1-2 rows of densely packed papillae.(Plate 3, figure F, Plate 5, figure I).
Senna obtusifolia (L) Irwin & Barneby	Ovary densely pubescent; style distally incurved and sometimes a little dilated; stigma oblique, egg like stigmatic orifice is surrounded by row of tapering hairs different from the epidermal hairs (Plate 2, figure I, Plate 3, figure G, Plate 4, figure F, Plate 5, figure A).
Senna tora L.	Ovary appressed, hairy, stigma truncate, papillate, style deflects towards left or right, upper part of style completely incurved forming a loop; stigma with minute papillae surrounding circular stigmatic orifice (Plate 2, figure J, Plate 3, figure H, Plate 5, figure B).
Senna occidentalis (L) Roxb.	Ovary white, hairy, style tip incurved, stigma plumose, laterally hooded. Stigmatic orifice elliptical, surrounded by several rows of hairs (Plate 2, figure L, Plate 3, figure L, Plate 5, figures C, G).
Senna siamea (Lam.) Irwin & Barneby	Carpel surface shows sparsely dispersed trichomes toward the upper portion and densely dispersed trichomes along the ovary. Style stout glabrous, its tip incurved and deflects towards left or right and bear papillate stigma with elliptical orifice (Plate 2, figure K, Plate 3, figure K, Plate 4, figure H).
Senna sophera (L) Roxb.	Ovary hairy, style tip incurved, stigma plumose, laterally hooded. Stigmatic orifice elliptical, surrounded by several rows of hairs (Plate 5, figure D).
Senna sulfurea (DC. ex Collad) Irwin & Barneby	Ovary silky hairy; style 5-7 mm long deflexed toward left and right, stigma terminal punctiform stigmatic orifice, irregular, surrounded by sparsely dispersed hairs (Plate 3, figure I, Plate 5, figure E).
Senna surattensis Burm.	Stigma punctiform, style pubescent, ovary stipitate, silky hairy, style deflexed, stigmatic orifice irregular or concealed surrounded by beak forming papillae (Plate 3, figure J, Plate 5, figure F).
Cassia fistula L.	Carpel surface shows sparsely dispersed trichomes towards the upper portion and densely dispersed trichomes along the ovary, the style is deflexed, hollow and on its tip are horizontal papillae (with respect to axis). Stigma is sublateral with pyriform orifice with lateral slits, the fringing trichomes surrounding the pore are unspecialized (Plate 3, figure A, Plate 4, figure D).
Cassia javanica L.	Carpel surface is glabrous or rarely sparsely dispersed, hairs are present on the lower part. Ovary slightly stipitate, style deflects towards left or right, curved stigma with beak like papillae surrounding concealed stigmatic orifice (Plate 3, figure B, Plate 4, figure B).
Cassia nodosa Buch.-Ham ex Roxb.	The carpel surface shows sparsely dispersed trichomes toward the upper portion and densely dispersed trichomes along the ovary, the style surface is glabrous and deflects towards left or right, stigma sublateral with pilate papillae forming circular or concealed stigmatic orifice (Plate 3, figure C, Plate 4, figure C).
Cassia renigera Wall. ex Benth.	Ovary and style pubescent, stigma sub-terminal, style deflects towards left or right, incurved, concealed stigmatic orifice surrounded by finger like papillate hairs (Plate 3, figure D, Plate 4, figures A, G).
Cassia roxburghii DC	Carpel stipitate, its surface shows sparsely dispersed trichomes, glaucous towards the upper portion and along the ovary. Style deflects towards left or right, incurved stigma with minute beak forming papillae surrounding concealed stigmatic orifice (Plate 3, figures E, Plate 4, figure E).

tora) or subequal, unequal, reflexed (*C. fistula, C. javanica* and *C. roxburghii*). The colour of petals may be yellow, red (*C. javanica, C. renigera* and *C. roxburghii*), yellowish green, velvety green (*C. nodosa*). Corolla consists of mostly five free petals. The aestivation of corolla is ascending imbricate, except in *C. javanica* where quincuncial aestivation has been observed. The edges of adaxial or posterior petals are inside while those of the lateral ones are overlapping. The colour varies from yellow to pinkish white. *C. nodosa*, with velvety and green calyx, differs from closely related *C. javanica* which has smooth calyx having deep red under-surface. The petals of former are more sharply pointed at tips than in the latter. The leafy bracts at the base of the flower stalks are heart shaped in *C. javanica* while in *C. nodosa* they are narrow and lanceolate. The distinct clusters of flowers intermingled with foliage are characters which distinguish the flowering of *C. javanica* from *C. renigera*. *C. nodosa* and *C. javanica* are closely related and are characterized by the presence of lateral racemes. In *C. renigera*, racemes grow from the scars of fallen leaves and in *C.*

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Plate 1

A-F. Disposition of androecium and gynoecium in flowers. A. *Cassia roxburghii*. B. *Cassia nodosa*. C. *Cassia javanica*. D. *Cassia fistula*. E. *Senna occidentalis*. F. *Senna surattensis*. G. *Senna obtusifolia*.
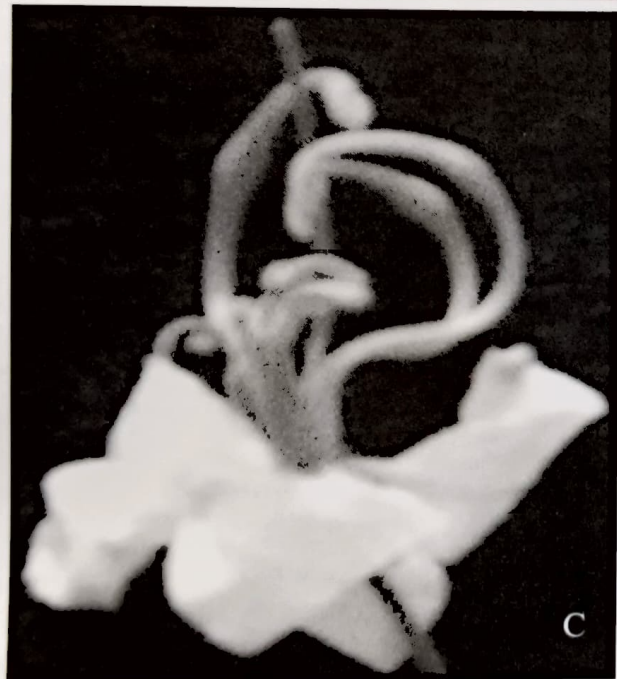
Plate 1

roxburghii, racemes grow in the axils of the leaves on the young twigs of the year. In some specimens of *C. fistula*, flattened leathery phylloclade like inflorescence axis has been observed. It is a deciduous tree and it becomes leafless for a very short time, or hardly at all, between March and May. The long pendulous racemes of large yellow bright flowers appear chiefly with the new leaves. The floral axis sometimes becomes flattened to increase the surface area for photosynthesis during the development of flower in absence of leaves. It may be considered as an ecological adaptation rather than constant feature (Plate 2, figure M). The androecium shows spectacular variation in the shape, size and morphology of stamens (Table 1). Considerable variation exists in stigmatic orifice and trichome morphology of carpel (Table 2). Carpenter bee (*Xylocopa pubescens*) is the frequent visitor on the flowers of tree species of both *Cassia* and *Senna* and black carpenter ants (*Camponotus pennysylvanicus*) seems to play an important role in pollination of herbaceous species of *Senna*.

RESULT AND DISCUSSION

Morphotaxonomical and adaptive implications of androecium

The species of *Cassia* and *Senna* are characterized by the presence of 10 stamens; either with polleniferous or sterile anthers of varying sizes, viz. long, medium and short anthers. The longer stamens are functional in pollination and the medium stamens are with fodder anthers (reward for pollinators), while the lower most (short) stamens are sterile or reduced to staminodes. The shape of different anthers, their structure and dehiscence are distinctive in different stamens and they are also distinctive in different species. The genus *Cassia* shows strong heteranthery and all

the 10 stamens of flowers are antheriferous but they show considerable differences in the length of filaments and size and form of anthers. The three tall and two short stamens are posterior lateral and constitute an outer antesepalous whorl, the four medium stamens and the short odd posterior stamens belong to inner antepetalous whorl. The three anterior stamens are the tallest and sigmoidally curved. The filaments of these stamens are many time longer than anthers and they are curved like the style, approximate the height of stigma. The filaments of stamens are nodulose in *C. nodosa* (Plate 1, figure B), *C. javanica* (Plate 1, figure C) and *C. renigera*, flat in *C. fistula* (Plate 1, figure D) and slender in *C. roxburghii* (Plate 1, figure A). The three posterior stamens are shortest and bear small anthers with or without pollen grains while the four centrally situated stamens are of medium height with fertile anthers. The anthers are dithecous and show variation in relative size, shape and mode of dehiscence. The anthers of outer antisepalous androecial whorl are elliptical, flattened with blunt apices and dehisces by incomplete and long ventral longitudinal slits. The anthers of inner antipetalous whorl are broad with round apex and basal spur. The anther dehiscence by sub-basal pores arises due to shrinking of spur in mature anthers (Venkatesh 1956a, b). Difference in the mode of dehiscence of anthers of outer and inner whorls is due to structural specialization of anther wall leading to development of stomium (Venkatesh 1956a). As the flowers mature, some changes occur in the disposition of various anthers which perhaps provides some significance in pollination (Venkatesh 1956b). In genus *Chamaecrista* (formerly sub-genus *Lasiorhegma*), all anthers of androecium are alike in form, structure and dehiscence though they differ in their relative size (Venkatesh 1956b).

Plate 2

A-H. Micro-morphology of anther showing pores and slits. A. *Senna occidentalis*. B. *Senna tora*. C. *Senna obtusifolia*. D. *Senna obtusifolia*. E. *Cassia roxburghii*. F. *Senna occidentalis*. G. *Cassia javanica*. H. *Cassia nodosa*.
I-K. Micro-morphology of entire carpel. I. *Senna obtusifolia*. J. *Senna tora*. K. *Senna siamea*. L. *Senna occidentalis*. M. *Cassia fistula* showing flattened floral axis. N. *Senna surattensis* showing anomalous apocarpous bicarpellary condition.
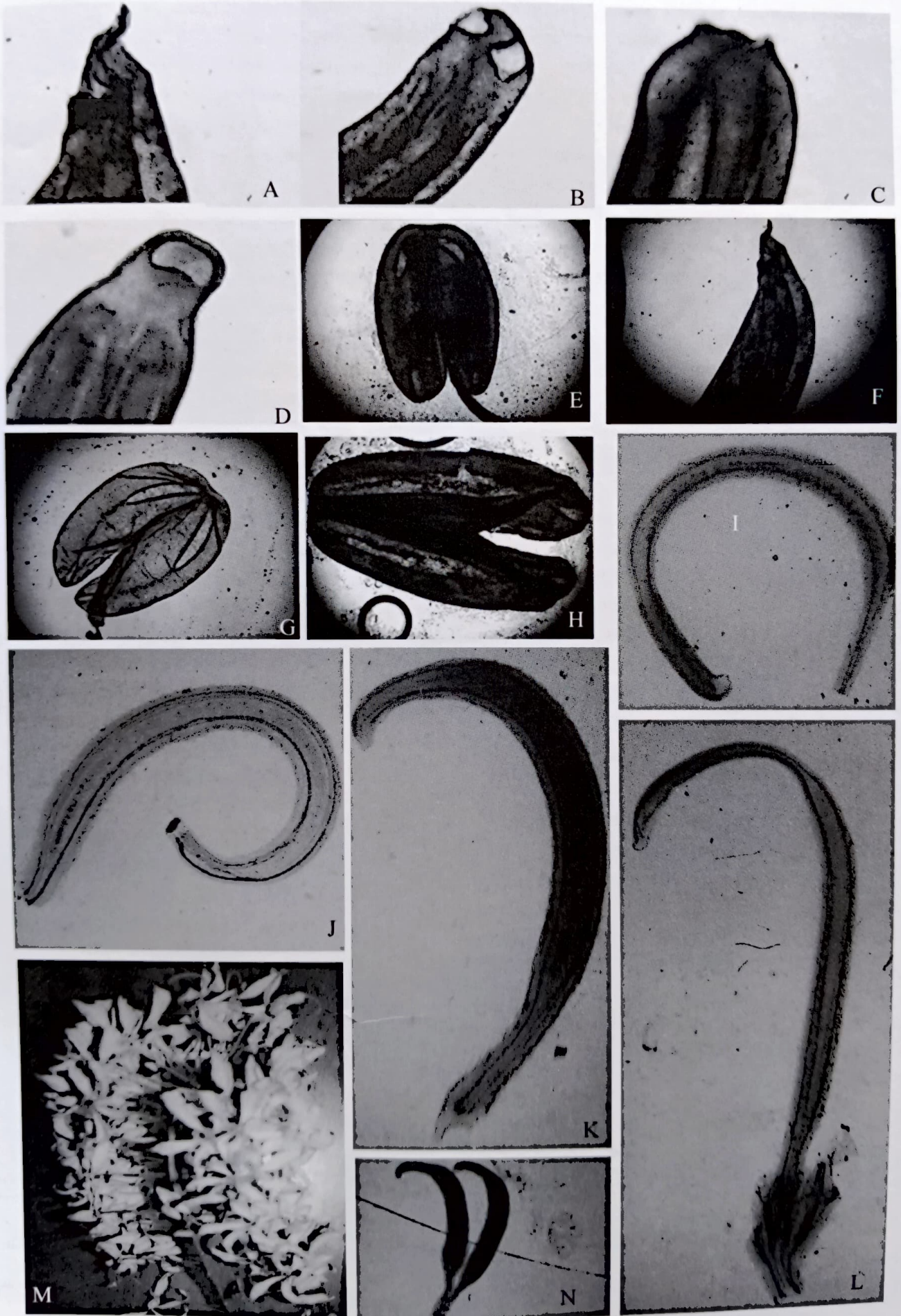
Plate 2

The genus *Senna* shows wider variation in androecium as compared to other two genera of sub-tribe Cassiinae. Except *S. sulfurea* and *S. surattensis*, the three posterior (adaxial) stamens of other species are sterile and reduced to pollen-less staminodes. In *S. occidentalis*, one of the anterior stamens of flower is also reduced to a staminode. The filaments of these stamens terminate into flattened blade-like structure that contains parenchymatous matrix instead of pollen sac (Venkatesh 1957). Though staminodes have lost their primary function of pollen production yet fulfill secondary floral functions of attracting pollinators and preventing explosive mechanism of pollination and autogamy (Walker-Larsen & Harder 2000). Despite greater degree of variation in androecial units, the genus *Senna* shows similarity in construction of anthers that are specialized for the purpose of dehiscence that occur by solitary or paired chinks or pores at the tips of the anthers. The androecial features can easily delimit the closely related species, viz. *S. obtusifolia* and *S. tora*. In the former, the three larger anterior anthers are narrowed shortly below their apex like the neck of a bottle while in the latter, the three larger anterior anthers are abruptly rounded at apex, not narrowed into a neck (Plate 2, figures B, D).

Thus the subtribe Cassiinae displays noticeable variation in androecium not only between *Cassia* and *Senna* but between the species of same genus. In Cassiinae, on the whole, there is evidence of progressive sterility and reduction of stamens of posterior side of both outer and inner whorls which finally lead to total suppression of stamens of inner whorl in *Chamaecrista absus* (Knuth 1904). In *Cassia*, the posterior smaller stamens bear pollen and show normal morphology. In *Senna*, except *S. surattensis* and *S. sulfurea*, three posterior stamens are reduced to sterile staminodes

which bear flattened blades in the place of anthers. Dehiscence of anthers by short slits or pore is the prominent feature of different species in Indian Cassiinae. Flowers do not secrete nectar and hence pollen grains are the main reward for the pollinators. When pollinators visit the flowers for the sake of pollen grains, they induce the vibration of stamens that causes rapid expulsion of pollen grains from the anther pore. The feeding anthers dust their pollen grains towards the anterior parts of pollinator and the pollinating anthers deposit their pollen on the part of the pollinator's body that comes in contact with receptive stigma. These pollen grains stick to the body parts of pollinating agents and transmitted to the stigma of other flowers during successive visits. This unique mode pollination mechanism is called buzz pollination or sonication (Buchmann 1983).

Morphotaxonomical and adaptive implications of gynoecium

In Cassiinae, gynoecium comprises single sickle shaped pistil consisting of short curved style and terminal or subterminal stigma. The carpel surface is covered with variously dispersed trichomes (Table 2, Plates 1-5). The semi-superior ovary is elongated, having variable number of campylotropous ovule attached on marginal placenta. The carpel features, viz. presence of trichome, shape of the stigma and position of stigmatic orifice, are variable in different species and bear infrageneric taxonomic significance. Efforts have been made to segregate *Cassia*, *Chamaecrista* and *Senna* using stigma characters such as pore size and variable morphology of fringing trichomes (Owens 1985, 1989, Owens & Lewis 1989). On the basis of stigma pore position and gross stigma anatomy, chambered and crateriform stigma have been recognized in different species of Cassiinae (Owens & Lewis 1989). The size of

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Plate 3

A-O. Micro-morphology of stigma under light microscope. A. *Cassia fistula* . B. *Cassia javanica*. C. *Cassia nodosa*. D. *Cassia renigera*. E. *Cassia roxburghii*. F. *Senna alata*. G. *Senna obtusifolia*. H. *Senna tora*. I. *Senna sulfurea*. J. *Senna surattensis*. K. *Senna siamea*. L. *Senna occidentalis*. M. *Cassia fistula*. N. *Cassia fistula*. O. *Cassia fistula*.
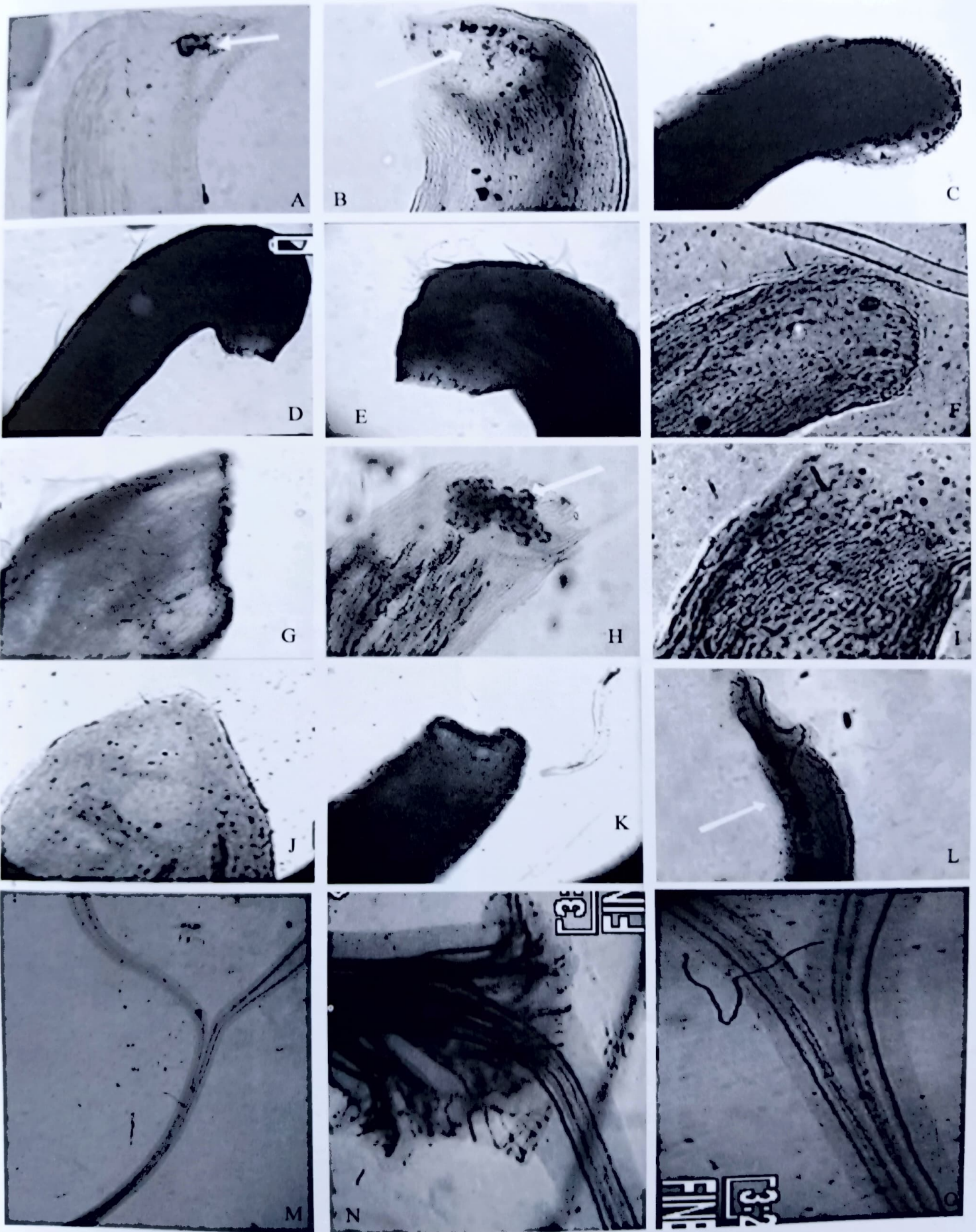
Plate 3

the pore or entrance to a narrow chamber varies in different species. The pore appears circular or oval but shape may vary both between and within the taxa. The stigmatic pore shape is variable in *Senna* as compared to *Cassia*. The micro-morphology of hairs around the stigmatic pore or crater helps to segregate *Cassia* from *Senna*. In *Cassia*, the hairs are morphologically similar to those of several *Senna* species but are fused to form tubular pore entrance, a character which clearly separates *Cassia* from *Senna*. In *Senna*, there occur considerable morphological variations with respect to hair number, size and shape but majority of the species can be recognized by stigma character unique to *Senna* (Plate 5).

Closely related nodded pink *Cassia* species can be distinguished by the carpel characteristics. In *C. javanica*, the carpel surface is glabrous or sparsely dispersed, hairs are present and stigmatic orifice is concealed surrounded by beak-like papillae (Plate 3, figure B, Plate 4, figure B). In *C. nodosa*, the upper part of carpel shows sparsely dispersed trichomes and the lower part shows densely dispersed trichomes. Stigma is sublateral with circular or concealed orifice surrounded by pilate papillae (Plate 3, figure C, Plate 4, figure C). In *C. renigera*, the ovary is pubescent, style is incurved, and stigma is subterminal with concealed orifice surrounded by finger-like papillae (Plate 3, figure D, Plate 4, figures A, G). The carpel micro-morphology of closely related *S. obtusifolia* and *S. tora* also play significant role in their taxonomic delimitation. In the former, the style is distally incurved and sometimes a little dilated, stigma is oblique and with oval orifice surrounded by row of tapering hairs (Plate 2, figure I, Plate 3, figure G, Plate 4, figure F, Plate 5, figure A). In the latter, the upper part of style is completely incurved forming a loop and stigma is truncate with circular

orifice surrounded by minute papillae (Plate 2, figure J, Plate 3, figure H, Plate 5, figure B). In few specimens of *C. fistula* and *S. surattensis*, bicarpellate apocarpous gynoecium has been observed as against monocarpellary gynoecium usually found in the species of Cassiinae and other leguminous species. The two carpels of gynoecium are fused by their receptacle but their ovary, style and stigma are free throughout the entire length and one of the carpel is fertile and the other is sterile. The two carpels receive the single vascular supply throughout the thalamus but two vascular traces emerge at the bifurcation of the receptacle (Plate 2, figure N, Plate 3, figures M-O) each supplying to individual carpel. Similar abnormality was also recorded in *C. surattensis* (Venkataraju & Pullaih 1986).

All the studied species of Casssiinae showed enantiostyly (Plate 1, figures A-G). The two anteriolateral large stamens are also inclined, each one to right and left of midline. Variations among the different species exist in the incurvature of style and position of stigmatic pore. The pistil is slightly arcuate in *S. siamea*, *C. roxburghii*, *C. javanica* and *C. nodosa*. The complete incurvature of the upper half of pistil forms a loop in *S. tora* and *S. obtusifolia* (Plate 2, figures I, J) resulting in close proximity of stigmatic orifice and anther pores.

The terminal or subterminal stigma is minute and papillate. Stigmatic orifice is surrounded by cuticularized hairs that may partially or entirely occlude the pore. Heslop-Harrison and Shivanna (1977) reported wet papillate stigma in Cassiinae. Owens (1985, 1989) recognized them as wet but non-papillate as the secretory materials are produced by cells surrounding the inner cavity and not by the papillae fringing it. The apex of the style lacks exposed receptive cell layer producing

Plate 4

A-H. SEM photomicrograph of carpel showing stigmatic opening, trichomes on the surfaces of style and guarding stigmatic orifice and slits. A. *Cassia renigera*. B. *Cassia javanica*. C. *Cassia nodosa*. D. *Cassia fistula*. E. *Cassia roxburghii*. F. *Senna obtusifolia* showing hairs on ovary surface. G. *Cassia renigera* showing surface of ovary. H. *Senna siamea* showing stigma feature.
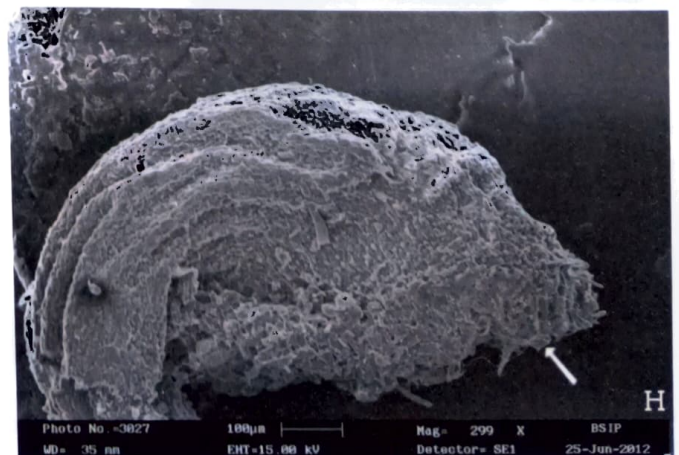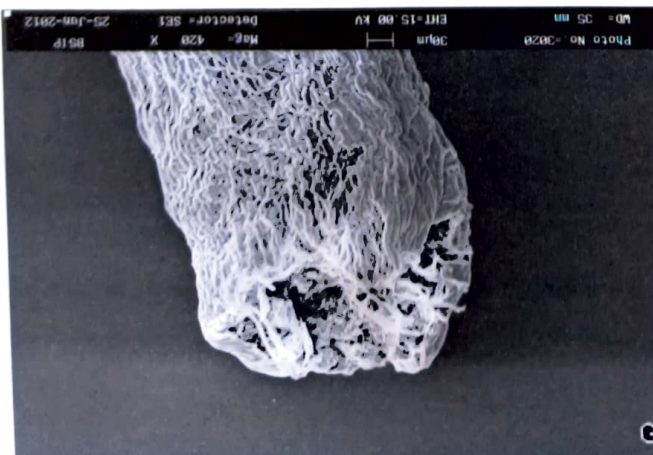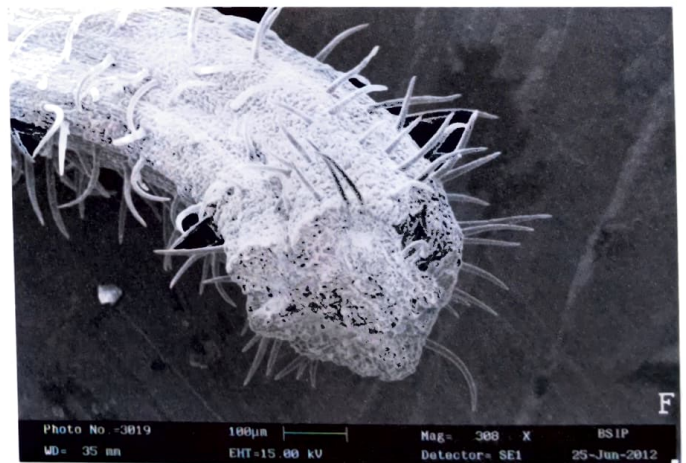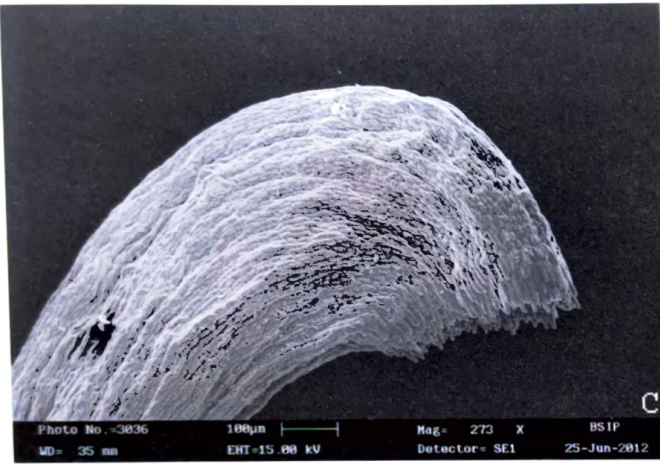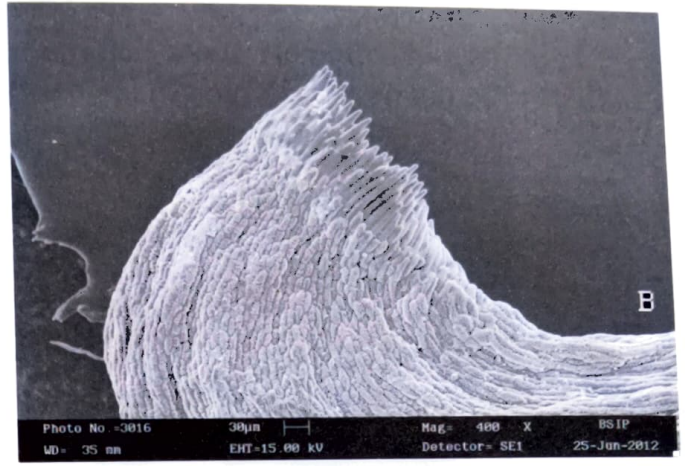
Plate 4

or storing free flowing secretion and capturing pollen grains, but has an orifice. Irrespective of their morphological specialization, the papillate cells surrounding the pore are not part of the tissue lining the cavity and producing the secretory material (Dulberger et al. 1994). Orifice at the end of stylar canal has also been reported in other plant species (Heslop-Harrison & Shivanna 1977, Schill et al. 1985). In most of these plants, the orifice at the end of style is surrounded or flanked by an exposed papillar or non-papillar layer on which pollen deposition and germination occur.

The fringing trichomes around pore are overlined with thick cuticle and are impermeable and they do not allow pollen germination. The germination of the pollen grains occurs in the secretion droplet in stigmatic orifice (Plate 3, figure H, Plate 5, figure A). Thus papillate stigma can be recognized both in *Cassia* and *Senna* in relation to the cells fringing the orifice and not to the receptive surface itself. Possibly the trichomes, concealing the orifice, are devised for protecting the stigmatic secretion.

In Cassiinae, it seems reasonable to recognize a special category of stigma in which secretory material is produced and accumulated within the cavity and is variably exposed at the orifice. The germination of pollen grains occurs in stigmatic cavity. This character shows its similarity with conifers and other gymnosperms. The distinct category of stigmatic orifices in Cassiinae reflects its specific adaptation to capture pollen grains and its subsequent exposure to secretary material retained in stigmatic cavity where pollen germination occurs.

ADAPTIVE POLLINATION STRATEGY

Vibratile pollination has been studied in more than 25 species belonging to the genus *Cassia* and *Senna* (Dulberger 1981, Delgado-Salinas & Sousa 1977, Gottsberger & Gottsberger 1988). Poricidal anthers predominantly prevail in the species of tropical climate that flowers during rainy season as an adaptation to protect the depletion of pollen grains during heavy rain (Harris 1905, Delgado-Salinas & Sousa 1977). In poricidal anthers, the opening of pores and pollen ejection is regulated by environmental conditions, particularly by ambient humidity (Gottsberger & Gottsberger 1988). In Indian Cassiinae, the flowering peaks coincide with the hot dry periods of pre- or post-monsoon to ensure effective pollination between especially evolved poricidal anthers and concealed stigma (Mishra & Srivastava 2010). The anthers open during dry weather of rainy season when pollinators become available. In small flowered *Chamaecrista* species, androecial differentiation is less pronounced and stamens contain fertile anthers and they are of the same length as the gynoecium (Gottsberger & Gottsberger 1988). The anther wall is soft and pollen grains can easily extrude out through the anther pores and innermost long and curved petals, called cucullus, direct the expulsion of pollen grains and its deposition on bee's body (Thorpe & Estes 1975, Costa et al. 2007). But in *Cassia* and *Senna* species, there is progressive tendency towards heteranthery. The androecia differ in form and function. The long pollinating stamens reach the height of stigma, median and central feeding stamens provide food and sitting platform for the pollinators and small stamens with sterile anthers or staminodes help the pollinators to cling to the flower during its visit. In *Cassia* and *Senna*, the flowers do not secrete nectar and in absence of nectar, pollen grains are the main reward for the pollinators and bright colouration of corolla and stamens are the primary attractants for the pollinators that do visit the flowers during dry periods. Thus, in absence

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Plate 5

A-H. SEM photomicrograph of carpel showing stigmatic opening, trichomes on the surfaces of style and guarding stigmatic orifice and slits. A. *Senna obtusifolia*. B. *Senna tora*. C. *Senna occidentalis*. D. *Senna sophera*. E. *Senna sulfurea*. F. *Senna surattensis*. G. *Senna occidentalis* showing ovary surface. I. *Senna alata*.
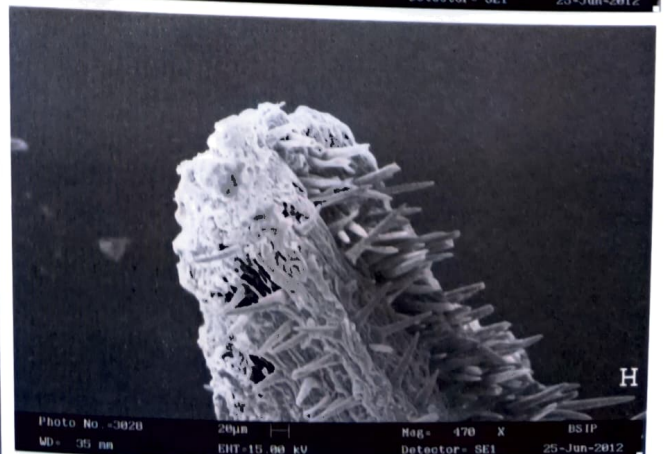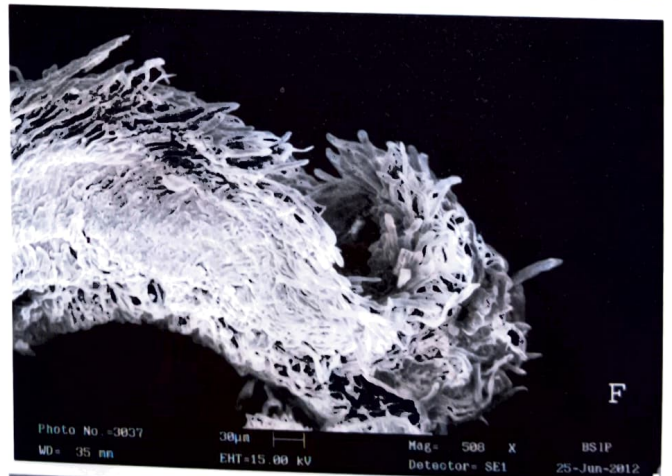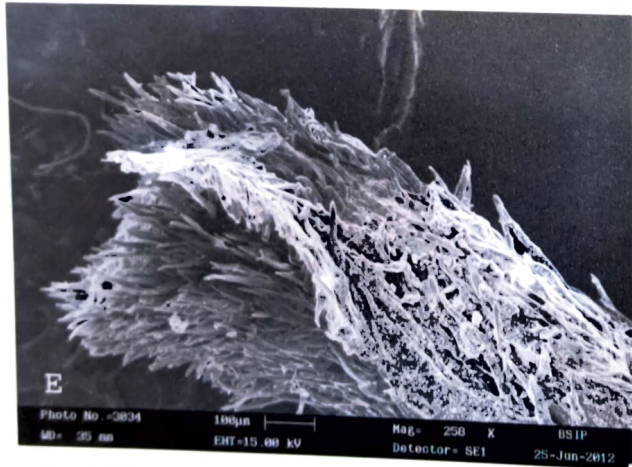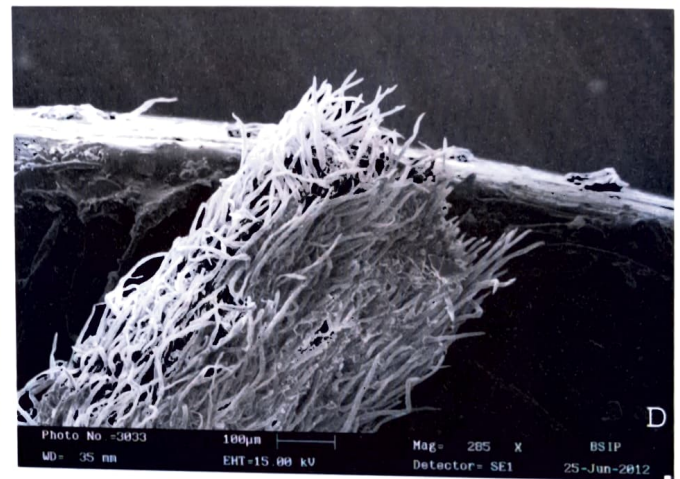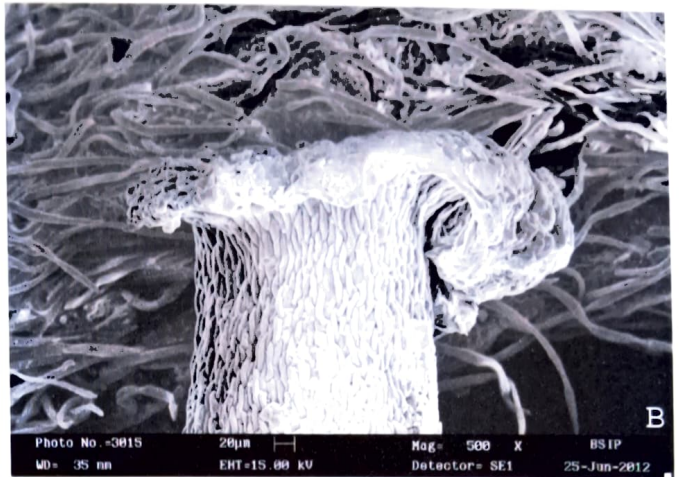
Plate 5

of nectar, these species offer feeding pollen grains to satisfy the interests of pollinator insect needed for effective transfer of male gametophyte on compatible stigma. Heteranthery has been evolved as a result of pollinator mediated selection and it helps in resolving the dilemma of fate of pollen grains as the food for pollinator as well as agent for transferring male gametophyte on receptive stigma (Vellejo-Marin et al. 2010) and it strongly supports the Darwin's hypothesis (1877) of division of labour among the stamens in flower (Luo et al. 2008). It is phylogenetically related with the absence of floral nectaries, buzz pollination and enantiostyly (Jesson et al. 2002). Enantiostyly, resulting into mirror image flowering, is a genetically determined phenomenon that promotes cross pollination in bee pollinated plants (Jesson et al. 2002). In *Cassia* species, both the abaxial pollinating stamens as well gynoecia are recurved to facilitate the simultaneous contact between anther pores and stigmatic orifice. Simultaneous vibrations of pollinating anthers and style by insect pollinator may result in ejection of pollen grains through anther pore and its subsequent suction by stigmatic cavity through specifically designed stigmatic orifice (Plate 1, figures A-D).

The androecium of *Senna* species are best adapted for buzz pollination. In these species, the stamens have short filaments bearing voluminous and poricidal anthers that eject pollen grains in response to sonication effect induced by the pollinators. When the pollinators visit the flowers and settle on the median central stamens, they induce the vibration of both feeding and pollinating anthers located on the ventral and dorsal side of its body, respectively. The vibration induces sonication effect on the poricidal anthers resulting in expulsion of pollen grains through the pores of the anthers and their deposition on the body surfaces of pollinator insects. During the course of vibratile movement of stamens, some vibrations get transmitted to adjacently placed curved and flexible style. The deflexed style vibrates like tuning fork in response to vibration caused by

pollinator. The stigmatic secretion, retained in stigmatic cavity, oozes out through stigmatic orifice on the surface of minute stigma located on the distal end of elongated style. The vibrating stigma and pollinating anthers simultaneously touch the dorsal surface of visiting pollinator but on opposite side. When the pollinator visits the flowers of opposite morph, its dorsal body surface is dusted with pollen grains during its previous visit comes in touch with stigma of the flower of opposite morph. The deflected style vibrates when it touches the insect body and as a result stigmatic fluid oozes out on the surface of stigma. The pollen grains from insect body get stick to the stigmatic secretion and get sucked inside the stigmatic cavity. The pollen grains germinate in the stigmatic cavity. In *S. obtusifolia* and *S. tora*, where the stamens are slightly differentiated and do not accompany the length of gynoecium, the deflected style forms loop to approximate the anther pores with stigmatic orifice (Plate 2, figures I-J).

Thus in Cassiinae, vibratile pollination undoubtedly prevails, in addition to enantiostyly and minute stigma with orifice. The androecia as well as gynoecia are better constructed and adapted for buzz pollination. Both heteranthery and enantiostyly evolved in Cassiinae to approximate the position of pollinating anthers and stigma of opposite floral morphs during the visits of insect. The bending of style in these species results in close proximity of stigma opening to anther pores and facilitates effective pollination. Thus combination of convergent characteristics, found in the members of these taxa, constitute pollination syndrome (Dulberger 1981). The arching of lower anthers apparently lends flexibility to anthers during vibrations of visitor and thus assists the ejection of pollen through the pores (Van der Pijl 1954, Dulberger 1981).

The trichomes fringing the stigmatic orifice hairs are stiff, thick walled and cuticularized and may act somewhat as fine comb for removing the pollen from the bee's body as also suggested

by Owens and Lewis (1989). The cuticular ornamentation of hair surface may help in retention of secretion droplets (Owens 1985) and may help in protection of the stigma from the mandibles of the bees (Dulberger 1981, Delgado-Salinas & Sousa 1977). Dulberger and Ornduff (1980) suggested that enantiostyly in *Cassia* species promotes out breeding and geitonogamy. Interspecific pollen transfer may be particularly critical in *Cassia*, where species examined proved to be self-compatible (Dulberger 1981, Owens 1985). Thus in Cassiinae, morphological and functional differentiation of anthers, specific orientation of pollinating and feeding anthers on surface of the pollinators, deflection of style towards left and right of median plane, minute internal stigma, concealed stigmatic cavity, poricidal anther dehiscence induced by visit of pollinators together constitute specific pollination strategy promoting insect mediated economic pollination, out breeding or geitonogamy. The arching of stamens provides flexibility to the anthers during the visit of pollinator and thus assists in ejection of pollen grains through pores. Vibration produced by pollinators promotes pollination through two-fold mechanism of ejection of pollen grains from anther and their subsequent absorption inside internal stigma by suction mechanism. Thus combination of convergent characters of morphological and functional differentiation of androecium and enantiostyly and internal stigma together constitute pollination syndrome to facilitate effective buzz pollination in species of sub-tribe Cassiinae.

CONCLUSION

It can be inferred from the present studies that Indian Cassiinae shows spectacular variation in form and function of androecium to facilitate effective pollination between specifically evolved androecium and gynoecium. Some of the micro-morphological features of stamens and carpel that have been evolved as adaptive structures also bear infrageneric taxonomic implications. Zygomorphism of flowers, elongation of gynoecia, long and curved style, enantiostyly, minute internal stigma with stigmatic orifice, heteranthery, poricidal anther dehiscence have been evolved to ensure efficient and economic pollination and to promote out breeding and geitonogamy.

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