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Significance of seed trichome micro-morphology in systematic treatment of *Abelmoschus-Hibiscus* complex

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Abstract

Indian sub-continent is rich in genetic diversity of various species of genus *Abelmoschus* and *Hibiscus*. Several of these species exist in wild, semi-wild and cultivated forms due to favorable eco-geographical conditions. Seed morphology (especially trichome structure) of Malvaceae members is known to be diverse, but variation patterns have never been critically studied in these complex genera. In the present paper usefulness of this important character for the generic differentiation between *Abelmoschus* (13 species) and *Hibiscus* (10 species) using scanning electron microscopy of seed has been described. Primary generic differentiation has been derived using basic seed trichome morphology i.e. unicellular (un-fused) and multi-cellular (fused) which was spiral or non–spiral, spread evenly or localized in all the studied taxa. The presence of unicellular and un-fused trichomes on seed coat surface in the *Abelmoschus* species provides an additional and significant micro-morphological differentiation to delineate the boundaries between genus *Abelmoschus* and *Hibiscus*.

Key words: *Abelmoschus*, generic differentiation, *Hibiscus*, micro-morphology, seed trichome, SEM, Abelmoschus-Hibiscus complex

Introduction

Heywood (1971) and Cole and Behnke (1975) drew attention to the importance and impact of scanning electron microscopy (SEM) in solving systematic problems in various critical species and genera of angiosperms. SEM studies have been made primarily on seeds, trichomes and pollen ornamentation with respect to study the species or sub-species delimitation and differentiation. According to Barthlott (1981) epidermal and seed surface features are more stable and seem to reflect genetic-phylogenetic differences in the plants concerned. The significance of SEM study lies in the fact that plant epidermis is thick and most stable (least affected by environment), covers almost all the plant parts. Therefore, during last few decades features of seed micro-morphology have been studied and reported to be useful to resolve taxonomic problems in various critical and overlapping taxonomic groups (Shavvon et al. 2012; Bona 2013; Azevedo et al. 2019; Tavakkoli and Assadi 2019; Kaya et al. 2019). The singular or multicellular epidermal hairs, known as trichomes have several functions in plants which include defense against insects, production of chemicals which deter insect and herbivores from feeding. Trichomes have a role in secretion of nectar, resins, mucilage, terpens etc in several of the plant species. Several type of trichomes have been reported and their variations studied and their utility in relation to plant taxonomy have been well demonstrated (Sudhakaran and Ganapathi 1993).

Family Malvaceae, commonly known as 'mallow family' comprises various economically important genera such as *Gossypium* (Cotton), *Corchorus* (Jute), *Hibiscus* (Kenaf) and *Alcea*, *Malva* (ornamentals importance) in tropical and subtropical regions (Sivarajan and Pradeep 1996) and therefore, has attracted great interest of plant systematists. Apart from these, genus *Abelmoschus* (Malvaceae) is very important due to its tasty, mucillagenous, nutritionally valuable, immature fruits bearing species *A. esculentus* (L.) Moench. Species of *Abelmoschus* exist in wild, semi-wild and cultivated forms, reflecting the vast ecological adaptation of the genus to different eco-

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geographical conditions of Indian sub-continent. Species of *Abelmoschus* including the economically important species *A. esculentus* show wide range of distribution in India, from Central Himalayan regions ((Velayudhan and Upadhya 1994; Negi and Pant 1998) to the hills of Western Ghats (Velayudhan and Upadhya 1994) and Southern part of India (Velayudhan et al. 1996). Similarly, the genus *Hibiscus* is also widespread; it comprises about 200 annual and perennial species. The genus is represented in the Indian flora by more than 10 species; most of them are in the peninsular India (Velayudhan and Upadhya 1994).

As originally conceived by Linnaeus (Linnaeus 1753, 1764) the genus *Hibiscus* encompassed all the capsular-fruited "mallows", except for the cottons. Over the time, botanists have differently divided *Hibiscus* into sections and segregated genera with distinctive flower or fruit characteristics (DeCandolle 1824). Taxonomy of *Abelmoschus* has complex history for the generic status and remains a matter of controversy for long. Firstly, *Abelmoschus* was evaluated under the genus *Hibiscus* L. as section by Linnaeus (1753). On the basis of capsule features, Medikus (1787) proposed to raise this section to the rank of a distinct genus. On the contrary, some taxonomists like Masters (1874), Prain (1903) and Dunn (1915) did not accept taxonomic treatment of Medikus and therefore, followed the view of De Candolle (1824). Hence, they accepted *Abelmoschus* as section of *Hibiscus*. Subsequently, Hochreutiner (1924) accepted taxonomic treatment of Medikus and raised the section *Abelmoschus* to the generic level on the basis of peculiar calyx configuration. Still taxonomic treatment of Medikus is valid and used in contemporary literature (Van Borssum-Waalkes 1966; Paul and Nair 1988; Vredebregt 1991; Sivarajan and Pradeep 1996; John et al. 2012; Sutar et al. 2013).

Among the various characters of plants, trichomes are widely distributed over the vegetative (stem, leaf) and reproductive parts (calyx, seed) of plants in Malvaceae. Sivarajan and Pradeep (1996) and Salah and Naggar (2001) reported that seed coat sculpturing pattern play significant role in generic differentiation among Malvaceaeous genera. Mwachala (1995) observed the micro-morphology of seed trichomes in *Hibiscus* section *Furcaria* and reported the structure of trichomes as unique, *i.e.* unicellular hairs form a scale like structure. On the account of generic differentiation Nwachukwu and Mbagwu (2008) provided anatomical characters of root and leaf to distinguish *A. esculentus* from *H. rosa-sinensis*. Additionally, Salah and Naggar (2001) have demonstrated the utility of hair characteristics (such as hair bases presence or absence, shape of hair bases) to distinguish the genus *Abelmoschus* from *Hibiscus*. However, their sampling size was too low (only 1 to 2 species of each genus) to draw any strong conclusion for the generic differentiation of *Abelmoschus* from *Hibiscus*.

Therefore, present study has been undertaken comprising 13 important species of *Abelmoschus* and 10 species of *Hibiscus* to assess the taxonomic significance of seed trichomes micro-morphology in the generic differentiation of *Abelmoschus* from *Hibiscus*.

Materials and methods

Scanning electron microscopy (SEM) studies were conducted on different species of *Abelmoschus* and *Hibiscus* to assess the taxonomic significance of variation in seed trichomes. Exploratory surveys were undertaken in different localities of India during the year 2008-12 to collect the seed material from naturally growing plants for the studies (Table 1). Floras and published literature were consulted for the correct identification of collected plant samples (Paul and Nair 1988; Sivarajan and Pradeep 1996). In the present article, for the generic circumscription and species treatment of *Abelmoschus* and *Hibiscus*, classification followed by Medikus (1787), Hochreutiner (1924), Van Borssum-Waalkes (1966) and IBPGR (1991) was used. Only 30 mature and healthy seeds for each true species were taken for further investigation.

For SEM investigations, the dried seeds were thoroughly cleaned and directly fixed on SEM specimen stubs with the help of double adhesive carbon tape and thereafter coated with thin film of gold using JEOL Fine Coat Ion Sputter (JEOL, JFC 1100). Specimens were examined using the Scanning Electron Microscope (JEOL, Japan, JSM 840A) mostly maintained at accelerating potential voltage of 10Kv. Digital photographs were taken using the attached camera and analysis for micro-morphology of surface and trichome structure was undertaken.

Results and discussion

Genus *Abelmoschus* is critical example of taxonomic ambiguity where unresolved status of many species, uncertainty in species number, polyploid nature, overlapping morphological characters and unknown phylogeny still exists. Recently, plant species have

Table 1. Detailsof specimen used in the study of seed trichome micromorphologyof *Abelmoschus*and *Hibiscus*

S.No.	Species	Status	Accession/ collector no.	Place of collection (Latitude/longitude)	Altitude (m)
	***Abelmoschus* species**				
1.	*A. esculentus* (L.) Moench.	Cultivated	Pusa Sawani	NA	NA
2.	*A. caillei* (A.Chev.) Stevels	Cultivated	IC587017	N5^{o} 26.860'/E20^{o} 88.221'	1012
3.	*A. moschatus* Medik. subsp. *moschatus*	Wild	IC140986	N8^{o} 38.999'/E77^{o} 03.698'	124
4.	*A. moschatus* Medik. subsp. *tuberosus* (Span.) Borss.	Wild	IC324070	N19^{o} 17.265'/E77^{o} 30.977'	487
5.	*A. tuberculatus* Pal & Singh	Wild	IC90359	N19^{o} 24.909'/E78^{o} 03.337'	432
6.	*A. ficulneus* (L.) Wight & Arn.	Wild	IC141001	N15^{o} 30.040'/E74^{o} 59.587'	644
7.	*A. crinitus* Wall.	Wild	BYM/10/2011	N19^{o} 43.478'/E78^{o} 17.201'	470
8.	*A. manihot* (L.) Medik. subsp. *manihot*	Wild	TCR 2303	N16^{o} 40.857'/E74^{o} 12.759'	569
9.	*A. manihot* (L.) Medik subsp. *tetraphyllus* (Roxb. ex Hornem.) Borss. Waalk. var. *tetraphyllus*	Wild	IC141019	N23^{o}34.630'/E78^{o} 33.261'	1828
10.	*A. manihot* (L.) Medik.subsp. *tetraphyllus* (Roxb. ex Hornem.) Borss. var. *pungens* (Roxb.) Hochr.	Wild	NMB 2933	N19^{o} 11.795'/E73^{o} 42.307'	904
11.	*A. angulosus* var. *grandiflorus* Thwaites	Wild	IC203863	N12^{o} 26.429'/E75^{o} 39.666'	694
12.	*A. enbeepeegearense* John et al.	Wild	JRN/09/25	NA	NA
13.	*A. palianus* Sutar et al.	Wild	SUA54	NA	NA
	***Hibiscus* species**				
14.	*H. micranthus* L.f.	Wild	IC383715	NA	NA
15.	*H. reenarayanianus* Anil Kumar et al. N. Ravi	Wild	IC545110	NA	NA
16.	*H. acetosella* Welw. ex Hiern.	Cultivated	EC571290	NA	NA
17.	*H. radiatus* Willd.	Cultivated	IC427640	NA	NA
18.	*H. vitifolius* Linn.	Cultivated	IC333573	NA	NA
19.	*H. furcatus* Roxb. ex DC.	Wild	IC471492	NA	NA
20.	*H. calyphyllus* Cav.	Wild	EC571298	NA	NA
21.	*H. cannabinus* Linn.	Cultivated	IC342608	NA	NA
22.	*H. sabdariffa* Linn.	Cultivated	IC417701	NA	NA
23.	*H. surattensis* Linn.	Wild	IC333505	NA	NA

NA = Not available

been considered as the central units of ecological and evolutionary studies, and therefore, the identification of boundaries among closely related species was an essential target of current systematic studies (Petit and Excoffier 2009). In this context, seed trichome characters of thirteen *Abelmoschus* and ten *Hibiscus* species were thoroughly surveyed in order to identify distinctiveness among them using scanning electron microscopy. Prominent type of trichomes and their characteristics among the species studied have been summarized in Table 2. Of which selected SEM micrographs of trichome types are presented in Figs. 1, 2 and 3.

Trichome distribution on the seed surface shows considerable variation among *Abelmoschus* and

Table 2. Comparisons of seed trichome characteristics among Abelmoschus and Hibiscus species as observed by SEM

S.No.	Species	Seed appearance	Characteristic of trichomes, if present	Trichome type
1.	*A. esculentus*	Glabrous	Deciduous trichomes: hairs drop down in the due course of time and remain as remnants on concentric rows, distance between rows ranges from 90-220 µm.	Type I
2.	*A. caillei*	Glabrous	-do-	Type I
3.	*A. moschatus* subsp. moschatus	Glabrous	-do-	Type I
4.	*A. moschatus* subsp. tuberosus	Glabrous	-do-	Type I
5.	*A. tuberculatus*	Pubescent	Unicellular, spiral, thin, acute and somewhat deflected.	Type II-A
6.	*A. ficulneus*	Pubescent	Unicellular, spiral, thin, acute, longer than A. tuberculatus	Type II-A
7.	*A. crinitus*	Glabrous	Instead of trichome smooth protuberance (warts) present in concentric rows on entire seed surface	Type III
8.	*A. manihot* subsp. manihot	Pubescent	Unicellular, non-spiral, acute with bulbous stalk	Type II-A
9.	*A. manihot* subsp. tetraphyllus var. tetraphyllus	Pubescent	Unicellular, spiral, acute with bulbous stalk	Type II-A
10.	*A. manihot* subsp. tetraphyllus var. pungens	Pubescent	Unicellular, non-spiral, acute with bulbous stalk.	Type II-A
11.	*A. angulosus* var. grandiflorus	Pubescent	Unicellular, non-spiral, acute with bulbous stalk, deflect upto 180° .	Type II-A
12.	*A. enbeepee-gearense*	Glabrous	Deciduous trichomes.	Type I
13.	*A. palianus*	Pubescent	Unicellular, non-spiral, erect, shorter than A. angulosus.	Type II-A
14.	*H. micranthus*	Glabrous	Multi-cellular, unfused and localized, silky thread like appearance,	Type II-B
15.	*H. sreenaraya-nianus*	Pubescent	No trichomes were seen	Type III
16.	*H. acetosella*	Glabrous	3 to 6 hairs fused, appressed, narrowly ovate, scale like appearance	Type II-B
17.	*H. radiatus*	Glabrous	8 to 10 hairs fused, appressed, broadly ovate, acute apex fully bent.	Type II-B
18.	*H. vitifolius*	Glabrous	3 to 6 hairs fused and fully covered with wax like deposition.	Type II-B
19.	*H. furcatus*	Pubescent	No trichomes were seen	Type III
20.	*H. calyphyllus*	Glabrous	3 to 4 hairs fused, hair length uniform, appressed, narrowly ovate, apex slightly bent	Type II-B
21.	*H. cannabinus*	Glabrous	8 to 9 hairs fused with uneven length, broadly ovate, apex bent towards seed coat	Type II-B
22.	*H. sabdariffa*	Glabrous	3 to 5 hairs partially fused and appressed, hairs comparatively long, acute.	Type II-B
23.	*H. surattensis*	Glabrous	2 to 3 hairs partially fused, hairs comparatively long and acute.	Type II-B

Hibiscus taxa, which has also been easily noticeable using light microscopy. Structure of trichomes shows significant variations among the studied species, and therefore, affords diagnostic characters in generic differentiation of *Abelmoschus* and *Hibiscus*. On the basis of structural variability of trichomes, the species could be clearly categorized into three main groups: Type I- Deciduous trichome; Type II- Persistent trichome and Type III- No Trichome.

Species of *Malvaceae* family exhibit several highly variable flower and fruit characters that have been used extensively in classification (Linnaeus 1753; Edlin 1935). Trichome structure and their type has received less attention than flower and fruit as a potentially informative character for delimiting genera in the family. Prantl (1891) was first to propose the use of trichome type to segregate the taxa at the tribal level. It was observed that all *Abelmoschus* species studied, namely, *A. esculentus*, *A. caillei*, *A. moschatus* subsp. *moschatus*, *A. moschatus* subsp. *tuberosus* and *A. enbeepeegearense* belonged to Type I (Fig. 1A-B). In this type, hairs drop down in the due course of time and remain as remnants on concentric

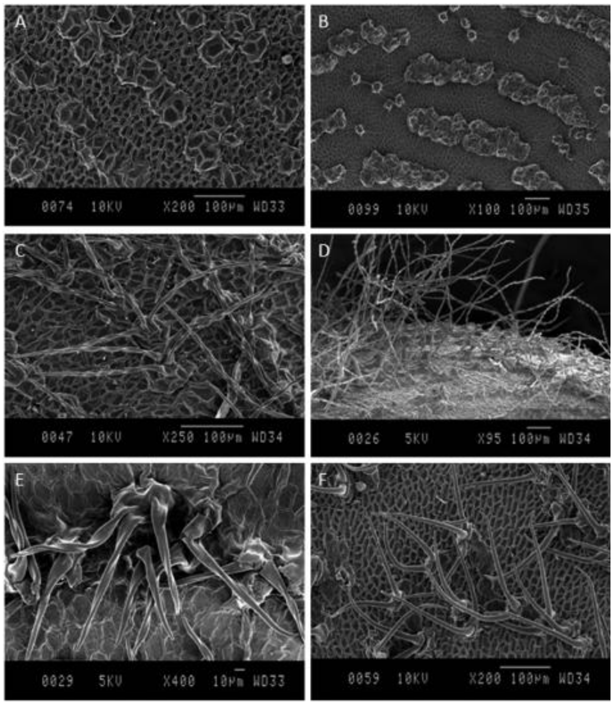

Fig. 1. SEM photographs of Type I. A - *A. esculentus*, B - *A. moschatus* subsp. *moschatus*; Type II-A. C - *A. tuberculatus*, D - *A. ficulneus*, E - *A. manihot* subsp. *tetraphyllus* var. *pungens*, F - *A. angulosus* var. *grandiflorus*

rows of seed coat with distance between rows ranging from 90 to 220 µm. Based on trichome structure, Type II was further divided into two sub-types, Type II-A: unicellular, un-fused and Type II-B: multi-cellular, fused. All *Abelmoschus* taxa (where trichome present) were featured in Type II-A (Fig. 1C-F) while all *Hibiscus* (where trichome present) were Type II-B (Fig. 2A-H). Similar observations have been made by Mwachala (1995) for some *Hibiscus* species. Morphologically, species with Type II-A trichomes with asymmetrical, spathaceous deciduous calyx can be easily distinguished from species having Type II-B trichomes by the features such as as campanulate or copular, and regular, accrescent calyx.

Interestingly, a single taxon of *Abelmoschus* (*A. crinitus*) and two taxa of *Hibiscus* (*H. sreenarayanianus* and *H. furcatus*) were characterized by Type III (Fig. 3A-C). However, *A. crinitus* can be distinguished by having smooth protuberance arranged in concentric rows instead of trichome, while seeds of *H. sreenarayanianus* and *H. furcatus* were found to be totally devoid of trichomes. Considering presence of these exclusive features, authors validate the taxonomic placement of *Abelmoschus* as a segregate

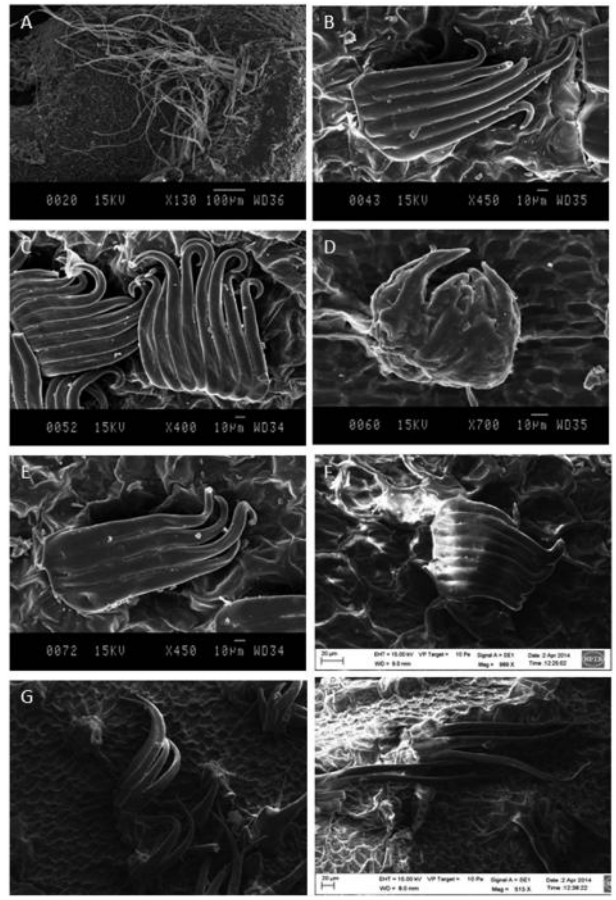

Fig. 2. SEM photographs of Type II-B. A- *H. micranthus*, B- *H. acetosella*, C- *H. radiatus*, D- *H. vitifolius*, E- *H. calyphyllus*, F- *H. cannabinus*, G- *H. sabdariffa*, H- *H. surattensis*

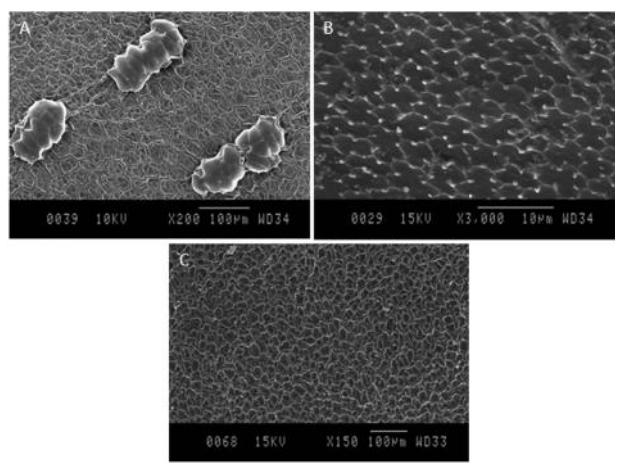

Fig. 3. SEM photographs of Type III. A- *A. crinitus*, B- *H. sreenarayanianus*, C- *H. furcatus*

genus of *Hibiscus* (Medikus 1787; Hochreutiner 1924). Similarly, trichome variation was used to delineate both at genus and species levels in Brassicaceae (Ancev

1991; Mullign 1995).

Besides the vital importance of trichomes in *Abelmoschus-Hibiscus* taxonomy, their evolutionary and ecological significance are also important. In fact, there seems to be a strong correlation between type of habitat and trichome distribution in a species. The deep presence of trichomes on seeds of *A. tuberculatus* is in partial agreement with Van Borssum-Waalkes (1966) treating it as a wild form of *A. esculentus*, since it generally grows along the roadsides and grassy slopes. Moreover, critical observations of seed micro-morphology in *A. tuberculatus* further supports the recognition of this distinct species as reported earlier by Pal et al. (1952) and Patil et al. (2018). However, detailed knowledge of ecological and genetic factors that are responsible for maintainance of distinct trichome structure among natural populations of *Abelmoschus* and *Hibiscus* would be of great importance in evolutionary biological studies of these genera.

In conclusion, the current study provides information on clear and comprehensive seed trichome distinctions which exist among species of *Abelmoschus* and *Hibiscus*. The present description of trichomes are an important and new addition to the descriptors being used currently for diversity characterization of these genera. Distinction and variation reported for seed trichome features would play an important role in taxonomy of family Malvaceae. These features would be useful in multidisciplinary studies of species relationships in these two important genera *Abelmoschus* and *Hibiscus*. Based on seed trichome structure, it is noteworthy that genus *Abelmoschus* is considerably distinct from *Hibiscus*. This study also validates the utility of micro-morphological features which can serve as a reliable approach for systematic studies as well as for effective characterization of *Abelmoschus* genetic resources.

Authors' contribution

Conceptualization of research (SKM, KVB); Designing of the experiments (SKM, PP, KVB); Contribution of experimental materials (KVP, PP, SKM); Execution of field/lab experiments and data collection (PP, SKM); Analysis of data and interpretation (SKM, PP, KVP); Preparation of manuscript (PP, SKM, KVB).

Declaration

The authors declare no conflict of interest.

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