Nordic Journal of Botany 33: 696–707, 2015
doi: 10.1111/njb.00771, ISSN 1756-1051

Subject Editor and Editor-in-Chief: Torbjörn Tyler. Accepted 27 January 2015

Taxonomic importance of seed macro- and micro-morphology in *Abelmoschus* (Malvaceae)

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Seed morphology of *Abelmoschus* is known to be variable, but patterns of variation have never been critically studied. We studied seed macro- and micro-morphological characters, including seed shape/size, seed coat pattern and trichome density/structure in multiple samples to evaluate the taxonomic significance of seed characters. Among the studied characters, seed shape and trichome structure were found to have major taxonomic importance and proved to be valuable characters for separating taxa. Two main seed types were present: seeds with deciduous trichomes and seeds with persistent trichomes. These characters offer significant evidence to the distinctness of certain species (*A. esculentus*, *A. moschatus* subsp. *moschatus*, *A. moschatus* subsp. *tuberosus*, *A. crinitus* and *A. angulosus*). Further, our results indicate that *A. moschatus* subsp. *tuberosus* should be maintained as a separate subspecies while *A. manihot* subsp. *tetraphyllus* var. *pungens* may be merged in *A. angulosus*. No significant intraspecific variation was observed, except in *A. esculentus*. We conclude that seed coat sculpturing and seed trichomes do indeed provide stable and diagnostic characters for many morphologically closely related taxa of *Abelmoschus* and that LM/SEM techniques can be useful in solving systematic problems and management of *Abelmoschus* genetic resources.

The genus *Abelmoschus* Medik. (Malvaceae) comprises nearly fifty species in the world (Charrier 1984), but it also contains many synonyms, misidentifications and taxa with unresolved status (Vredebregt 1991). Originally, *Abelmoschus* was treated as a section of *Hibiscus* L. by Linnaeus (1753). On the basis of capsule features, Medikus (1787) proposed to raise this section to the rank of genus. However, other taxonomists like Masters (1874), Prain (1903) and Dunn (1915) did not accept the taxonomic treatment of Medikus and preferred to follow De Candolle (1824). Hence, they accepted *Abelmoschus* as a section of *Hibiscus*. Subsequently, Hochreutiner (1924) accepted the taxonomic treatment of Medikus and raised the section *Abelmoschus* to the generic level on the basis of its peculiar calyx configuration. Still today the generic treatment of Medikus (1787) is valid and used in the contemporary literature (Van Borssum-Waalkes 1966, Paul and Nair 1988, Vredebregt 1991, Sivarajan and Pradeep 1996).

At the species level, Hochreutiner (1924) distinguished fourteen species while Van Borssum-Waalkes (1966) maintained only six species, including three cultivated [*A. moschatus* Medik., *A. manihot* (L.) Medik. and *A. esculentus* (L.) Moench.] and three wild [*A. ficulneus* (L.) Wight & Arn., *A. crinitus* Wall., and *A. angulosus* Wall.]. In contrast, Paul and Nair (1988) accepted seven species from India alone. Although nine species were maintained in the classification system adapted by the International Okra workshop (IBPGR 1991), most recently, two new additions i.e. *A. enbeepeegearense* J John, Scariah, Nissar, KV Bhat and SR Yadav and A. *palianus* Sutar, KV Bhat et SR Yadav have been made by John et al. (2012) and Sutar et al. (2013), respectively. At present, *Abelmoschus* comprises 7 species, 3 subspecies and 5 varieties in India.

The diverse agroclimatic conditions encompassing variable rainfall and soil regimes have promoted diversification of *Abelmoschus* in southeast Asia. The genus displays a variable habit, from annual to perennial, herbs to shrubs with leaves that are long petiolate, hastate or palmately lobed, pubescent or glabrous, and flowers that are white, dark yellow, pink to red (Sivarajan and Pradeep 1996, John et al. 2012, Sutar et al. 2013). The species of *Abelmoschus*, including the economically important species *A. esculentus* and *A. caillei* (A.Chev.) Stevels shows a wide range of distribution in India from the central Himalayan region (Velayudhan and Upadhay 1994, Negi and Pant 1998), hills of Western Ghats (Sivarajan and Pradeep 1996) to the southern part of India (Velayudhan et al. 1996). Some of the wild taxa also occur in north Australia, South America and Africa (IBPGR 1991). Recently, it has been stated that species of *Abelmoschus* are multi-purpose in utilization because of the exceptional medicinal and nutritional value of both seeds and leaves (Ngoc et al. 2008).

Heywood (1971) and Cole and Behnke (1975) drew attention to the importance and impact of scanning electron microscopy (SEM) in solving systematic problems. Features of the seed (macro- and micro-morphological) have been reported to be useful for the taxonomy in numerous plant groups (Minuto et al. 2006, Vural et al. 2008, Hamilton et al. 2008, Shavvon et al. 2012, Bona 2013). Sivarajan and Pradeep (1996) and Salah and Naggar (2001) recognized that seed coat sculpturing pattern play a significant role in species delimitation among malvaceaeous taxa.

However, the present study is the first comprehensive study on the seed micro-morphology in *Abelmoschus* and our aim has been to provide data from multiple samples to give detailed information about the pattern of variation in seed characters and their significance for solving taxonomic problems.

Material and methods

Germplasm augmentation and LM studies

Exploratory surveys were undertaken in different wild *Abelmoschus* localities in India during 2008–2013 to collect seed material from living plants for the present study. Specimens were identified by the fourth author and further confirmation was ensured with the help of information gathered from floras and published reports (Paul and Nair 1988, Bisht et al. 1995, Sivarajan and Pradeep 1996). The herbarium specimens were deposited in SUK. In the present article, for the generic circumscription and species treatment of *Abelmoschus*, we basically followed Medikus (1787), Hochreutiner (1924) and the IBPGR (1991) classification system. A total 105 accessions encompassing two cultivated, one semi-wild, ten wild and two newly described taxa were used in the present study (Table 1). Only mature and healthy seeds (30 seeds) from each accession were taken for further investigation. The dry seeds were thoroughly cleaned, and examined under a binocular stereoscopic microscope to study macro-morphological parameters, viz. shape, color and seed surface texture. Axis parameters were measured with the aid of an ocular micrometer placed in the binocular microscope.

Seed preparations for SEM

For SEM investigations, the dried seeds were directly fixed on specimen stubs with the help of double adhesive carbon tape, coated witha thin film of gold using JEOL Fine Coat Ion Sputter (JEOL, JFC 1100), and examined with scanning electron microscope (JEOL, JSM 840A) maintained at an accelerating potential voltage of 10 kV. The terminology used for description of seed macro- and micro-morphological characters is according to Barthlott (1981) and Stearn (1992).

Results

The seed morphological characters of the studied taxa of the *Abelmoschus* as shown by LM and SEM are elaborated in Table 2 and 3. Primarily, on the basis of trichome nature, two types of seeds were identified in the studied taxa of *Abelmoschus*:

Type I: seeds with deciduous trichomes

In this type, seed length/width ratio ranged between 1.08 and 1.40, seed color varied from light green to dark brown (Fig. 1A– F) and trichomes were deciduous, i.e. they drop down in the due course of time and remain only as remnants in concentric rows (Fig. 2A–F). The distance between these rows ranged between 90 and 220 μm. On the basis of seed shape, two subtypes could be distinguished in this group:

Subtype IA

Seeds typically globose-subglobose with light green to grayish color and a reticulate sculpturing pattern. The two cultivated species, *A. esculentus* (Fig. 3A–C) and *A. caillei* (Fig. 3D–F) had seeds of this type.

Table 1. Details of *Abelmoschus* specimens used in the present study.

Sr. no.	Taxon	Biological status	No. of analyzed accessions	Voucher no. of deposited specimen
1.	*A. esculentus* (L.) Moench.	Cultivated	22	SUA-28
2.	*A. caillei* (A.Chev.) Stevels	Cultivated	10	SRYA-89
3.	*A. moschatus* Medik. subsp. *moschatus*	Wild	13	SUA-32
4.	*A. moschatus* Medik. subsp. *tuberosus* (Span.) Borss.	Semi-wild	4	SUA-31
5.	*A. tuberculatus* Pal & Singh	Wild	7	SUA-59
6.	*A. ficulneus* (L.) Wight & Arn.	Wild	10	SUA-47
7.	*A. crinitus* Wall.	Wild	3	SUA-27
8.	*A. manihot* (L.) Medik. subsp. *manihot*	Wild	3	SUA-42
9.	*A. manihot* (L.) Medik subsp. *tetraphyllus* (Roxb. ex Hornem.) Borss. Waalk. var. *tetraphyllus*	Wild	10	SUA-35
10.	*A. manihot* (L.) Medik. subsp. *tetraphyllus* (Roxb. ex Hornem.) Borss. var. *pungens* (Roxb.) Hochr.	Wild	6	SPA-1
11.	*A. angulosus* var. *grandiflorus* Thwaites	Wild	5	SUA-40
12.	*A. angulosus* var. *purpureus* Thwaites	Wild	3	SUA-34
13.	*A. angulosus* var. *angulosus* Sivrajan and Pradeep	Wild	3	SUKA-11
14.	*A. enbeepeegearense* John et al.	Wild	4	SUKA-9
15.	*A. palianus* Sutar et al.	Wild	2	SRYA-54

Table 2. Comparisons of seed macro-morphological characteristics among *Abelmoschus* species under LM.

Sr. no.	Species	Length (mm)	Width (mm)	Length/ width ratio	Shape	Color	Hilum shape	Seed appearance
1.	*A. esculentus*	5.39 ± 0.23	4.99 ± 0.04	1.08	Subglobose	Light green	Broad ovate	Glabrous
2.	*A. caillei*	5.48 ± 0.05	4.80 ± 0.04	1.14	Subglobose	Greenish	Broad ovate	Glabrous
3.	*A. moschatus* subsp. *moschatus*	3.82 ± 0.03	2.95 ± 0.05	1.29	Flattened reniform	Brown	Rectangular	Glabrous
4.	*A. moschatus* subsp. *tuberosus*	3.73 ± 0.04	2.85 ± 0.06	1.30	Flattened reniform	Dark brown	Rounded	Glabrous
5.	*A. tuberculatus*	3.41 ± 0.04	3.44 ± 0.22	0.99	Globose	Light brown	Broad ovate	Pubescent
6.	*A. ficulneus*	3.09 ± 0.02	3.07 ± 0.02	1.00	Globose	Greenish	Ovate	Pubescent
7.	*A. crinitus*	3.92 ± 0.03	2.80 ± 0.03	1.40	Reniform	Dark brownish	Rounded	Glabrous
8.	*A. manihot* subsp. *manihot*	3.90 ± 0.05	3.39 ± 0.03	1.15	Subglobose	Light greenish	Rounded	Pubescent
9.	*A. manihot* subsp. *tetraphyllus* var. *tetraphyllus*	3.88 ± 0.05	3.41 ± 0.03	1.13	Subglobose	Light greenish	Rounded	Pubescent with golden hairs
10.	*A. manihot* subsp. *tetraphyllus* var. *pungens*	3.59 ± 0.02	2.78 ± 0.05	1.29	Subreniform	Dark brown	Triangular	Pubescent with golden hairs
11.	*A. angulosus* var. *grandiflorus*	2.99 ± 0.02	2.55 ± 0.03	1.17	Subglobose	Brown	Rounded	Pubescent
12.	*A. angulosus* var. *purpureus*	3.53 ± 0.03	3.31 ± 0.01	1.06	Subglobose	Dark brown	Rounded	Pubescent
13.	*A. angulosus* var. *angulosus*	3.71 ± 0.05	3.21 ± 0.05	1.15	Subglobose	Brownish	Rounded	Pubescent
14.	*A. enbeepeegearense*	3.70 ± 0.04	2.80 ± 0.06	1.32	Flattened reniform	Brown	Rounded	Glabrous
15.	*A. palianus*	3.55 ± 0.04	3.33 ± 0.02	1.06	Subreniform	Dark brown	Rounded	Pubescent

Subtype IB

This subtype differed from subtype IA by having a characteristic flattened reniform seed shape and brown to blackish colored seeds. In addition, some taxa, e.g. *A. moschatus* subsp. *tuberosus* and *A. crinitus*, showed an impressive seed coat pattern formed by storied reticulations, while the rest of the species of this subtype had a simple reticulate surface pattern. This subtype was represented by a morphologically diverse group of species including as *A. crinitus* (Fig. 3G–I), *A. moschatus* subsp. *moschatus* (Fig. 3J–L), *A. moschatus* subsp. *tuberosus* [(Span.) Borss, Fig. 3M–O] and *A. enbeepeegearense* (Fig. 4A–C).

Type II: seeds with persistent trichomes

The seeds of this type are almost covered with golden or white (Fig. 1G–O), spiral or non-spiral trichomes which are spread evenly or localized on the seed surface (Fig. 2G–N). The seed length/width ratio ranged between 0.99 and 1.40. On the basis of trichome structure and anticlinal wall features two subtypes could be distinguished:

Subtype IIA

Seeds of this subtype had golden, evenly spread trichomes which were long, thin and spiral, except for *A. manihot* subsp. *manihot* which had non-spiral trichomes. The anticlinal walls were more or less tightly joined in this subtype. This subtype comprises *A. ficulneus* (Fig. 4D–F), *A. tuberculatus* Pal & Singh (Fig. 4G–I), *A. manihot* subsp. *manihot* (Fig. 4J–L) and *A. manihot* subsp. *tetraphyllus* (Roxb. ex Hornem.) Borss. Waalk. var. *tetraphyllus* (Fig. 4M–O). The periclinal walls were found to be wrinkled in *A. tuberculatus*, while they were not observable in *A. ficulneus*. Most notably, the seed coat pattern was almost similar in *A. manihot* subsp. *manihot* and *A. manihot* subsp. *tetraphyllus* var. *tetraphyllus*.

Subtype IIB

Seeds of this subtype were characterized by brown to dark brown color, thick anticlinal walls and flat or concave periclinal walls (Table 3). Trichomes were found to be short and deflected 180° from a prominent bulbous base. This type was widely represented by *A. manihot* subsp. *tetraphyllus* (Roxb. ex Hornem.) Borss. var. *pungens* (Roxb.) Hochr. (Fig. 5A–C), *A. angulosus* var. *grandiflorus* Thwaites (Fig. 5D–F), *A. angulosus* var. *purpureus* Thwaites (Fig. 5G–I), *A. angulosus* var. *angulosus* Sivrajan & Pradeep (Fig. 5J–L) and *A. palianus* (Fig. 6A–C). Most notably, seeds of *A. palianus* showed great similarity with those of *A. angulosus* var. *purpureus* with respect to seed coat pattern.

Discussion

The genus *Abelmoschus* is a typical example of taxonomic ambiguity since many proposed species have an unresolved status, the number of species in the genus is uncertain, morphological characters are overlapping and the phylogeny is unknown. India is the country with the highest number of *Abelmoschus* species taxonomically accepted by Hinsley (2013). All the same, no systematic efforts have so far been attempted in India to generate the basic information needed for identifying species in *Abelmoschus*. Still, plant species are considered as the central units of ecological and evolutionary studies, and therefore, the identification of boundaries among closely related species is an essential target of current systematic studies (Petit and Excoffier 2009, Edlley et al. 2012). In this context, we thoroughly surveyed seed characters of fifteen *Abelmoschus* species using LM and SEM in order to identify diagnostic differences. As a result, it was concluded

Table 3. Comparisons of seed micro-morphological characteristics among *Abelmoschus* species as observed under SEM.

Sr. no.	Species	Trichome type	Trichome characteristics	Testa pattern	Epidermal cell	Anticlinal wall	Periclinal wall
1.	*A. esculentus*	Deciduous	Trichome remnants in concentric rows	Reticulate	Polygonal, compact	5−7 μm thick, raised, straight	17−21 × 7−12 μm wide, Slightly concave, waxy
2.	*A. caillei*	Deciduous	Trichome remnants in concentric rows	Reticulate	Tetra-pentagonal	6−10 μm thick, raised, straight	23−36 × 14−20 μm wide, slightly concave, wrinkle
3.	*A. moschatus* subsp. *moschatus*	Deciduous	Trichome remnants in concentric rows	Reticulate	Penta-hexagonal	2.8 μm thick, raised, straight	14−40 × 10−15 μm wide, flat, not smooth
4.	*A. moschatus* subsp. *tuberosus*	Deciduous	Trichome remnants in concentric rows	Storied reticulate	Penta-hexagonal	2.7 μm thick, raised, straight, overlapped	13−35 × 11−14 μm wide, slightly flat, not smooth
5.	*A. tuberculatus*	Persistent	Unicellular, spiral, thin, acute	Reticulate	Polygonal	2.5−4 μm thick, raised	18−27 × 14−17 μm wide, waxy, slightly concave
6.	*A. ficulneus*	Persistent	Unicellular, spiral, thin, acute	Reticulate-foveate	Polygonal	2.5−4 μm thick, tightly adjoined	21−28 μm wide, concave
7.	*A. crinitus*	Absent	Smooth protuberances in rows	Storied reticulate	Tetra-hexagonal	2−4 μm thick, overlapped	7−20 × 5−7 μm wide, slightly concave, wavy
8.	*A. manihot* subsp. *manihot*	Persistent	Unicellular, non-spiral, acute with bulbous stalk	Reticulate	Penta-hexagonal	7−14 μm thick, smooth, wavy	10−21 × 7−10 μm wide, slightly concave, smooth
9.	*A. manihot* subsp. *tetraphyllus* var. *tetraphyllus*	Persistent	Unicellular, spiral, acute with bulbous stalk	Reticulate	Tetra-pentagonal	7−14 μm thick, smooth, wavy	10−21 × 7−10 μm wide, slightly concave, smooth
10.	*A. manihot* subsp. *tetraphyllus* var. *pungens*	Persistent	Unicellular, non-spiral, acute with bulbous stalk	Reticulate	Polygonal	2.8−4.2 μm thick	21−28 × 14−21 μm wide, flat, not smooth
11.	*A. angulosus* var. *grandiflorus*	Persistent	Di–tri cellular, non-spiral, acute with bulbous stalk, deflect up to 180°	Reticulate	Tetra or pentagonal, elongate	2.5−3.7 μm thick, elongate, smooth, raised	12−25 × 8.7−14 μm wide, slightly concave
12.	*A. angulosus* var. *purpureus*	Persistent	Di–tri cellular, non-spiral, acute with bulbous stalk, deflect up to 180°	Reticulate	Tetra or pentagonal	2.8−4 μm thick, smooth, raised	17−23 × 10−14 μm wide, slightly concave
13.	*A. angulosus* var. *angulosus*	Persistent	Di–tri cellular, non-spiral, acute with bulbous stalk, deflect up to 180°	Reticulate	Polygonal	2.8−4 μm thick, wrinkled, raised	11−31 × 7−14 μm wide, concave
14.	*A. enbeepeegearense*	Deciduous	Trichome remnants in concentric rows	Reticulate	Penta-hexagonal	1.6 μm thick, raised, smooth	18−22 × 13−18 μm wide, flat
15.	*A. palianus*	Persistent	Unicellular, stiff, spiral only at apex	Reticulate	Tetra or pentagonal	2.5−3.7 μm thick, elongate, smooth, raised	17−23 × 10−14 μm wide, slightly concave

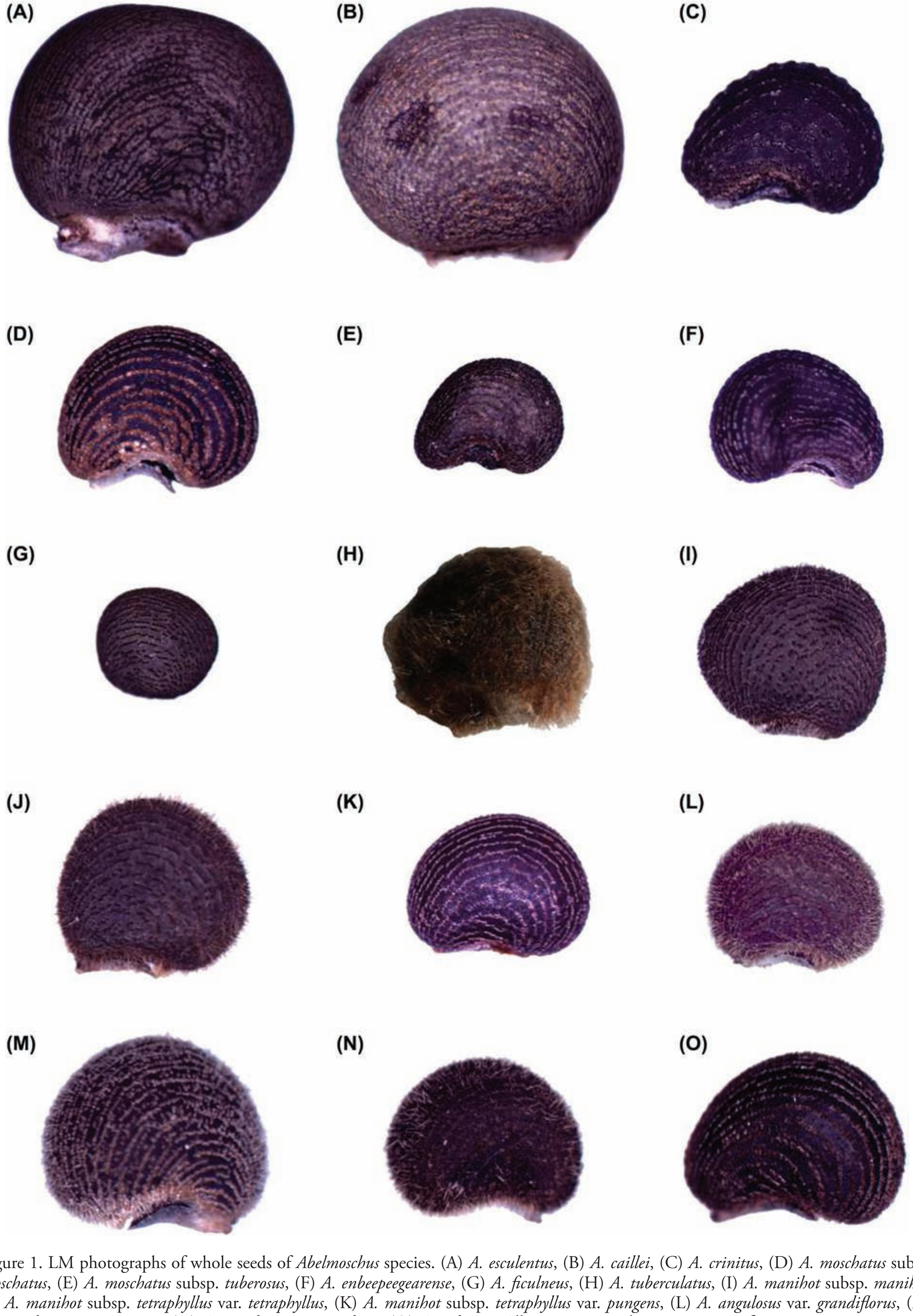

Figure 1. LM photographs of whole seeds of *Abelmoschus* species. (A) *A. esculentus*, (B) *A. caillei*, (C) *A. crinitus*, (D) *A. moschatus* subsp. *moschatus*, (E) *A. moschatus* subsp. *tuberosus*, (F) *A. enbeepeegearense*, (G) *A. ficulneus*, (H) *A. tuberculatus*, (I) *A. manihot* subsp. *manihot*, (J) *A. manihot* subsp. *tetraphyllus* var. *tetraphyllus*, (K) *A. manihot* subsp. *tetraphyllus* var. *pungens*, (L) *A. angulosus* var. *grandiflorus*, (M) *A. angulosus* var. *purpureus*, (N) *A. angulosus* var. *angulosus*, (O) *A. palianus*. All images were taken at 3 × magnification.

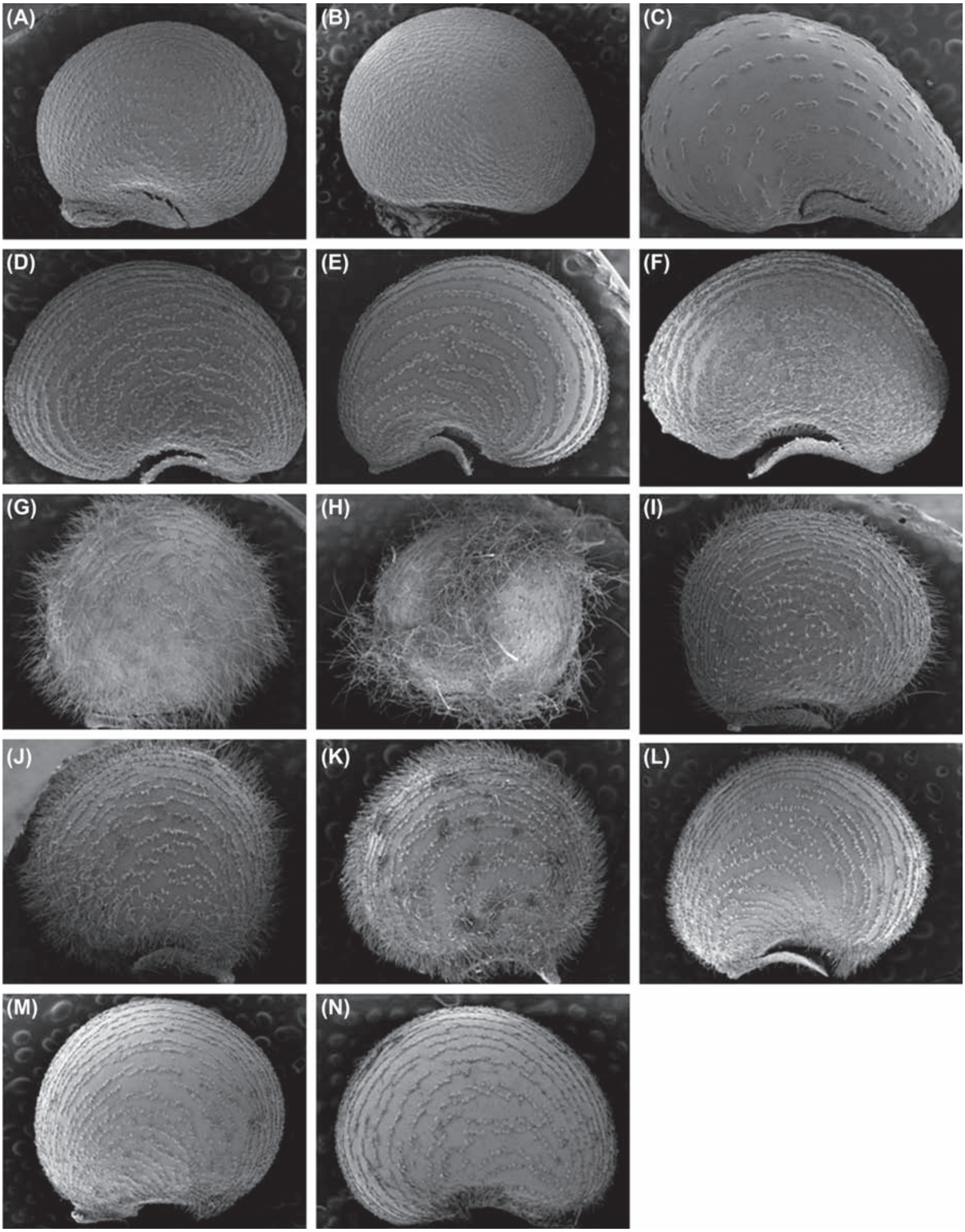

Figure 2. SEM micrographs of whole seeds of *Abelmoschus* species showing the variability in seed shape and surface topography. (A) *A. esculentus*, (B) *A. caillei*, (C) *A. crinitus*, (D) *A. moschatus* subsp. *moschatus*, (E) *A. moschatus* subsp. *tuberosus*, (F) *A. enbeepeegearense*, (G) *A. ficulneus*, (H) *A. tuberculatus*, (I) *A. manihot* subsp. *manihot*, (J) *A. manihot* subsp. *tetraphyllus* var. *tetraphyllus*, (K) *A. manihot* subsp. *tetraphyllus* var. *pungens*, (L) *A. angulosus* var. *grandiflorus*, (M) *A. angulosus* var. *purpureus*, (N) *A. angulosus* var. *angulosus*.

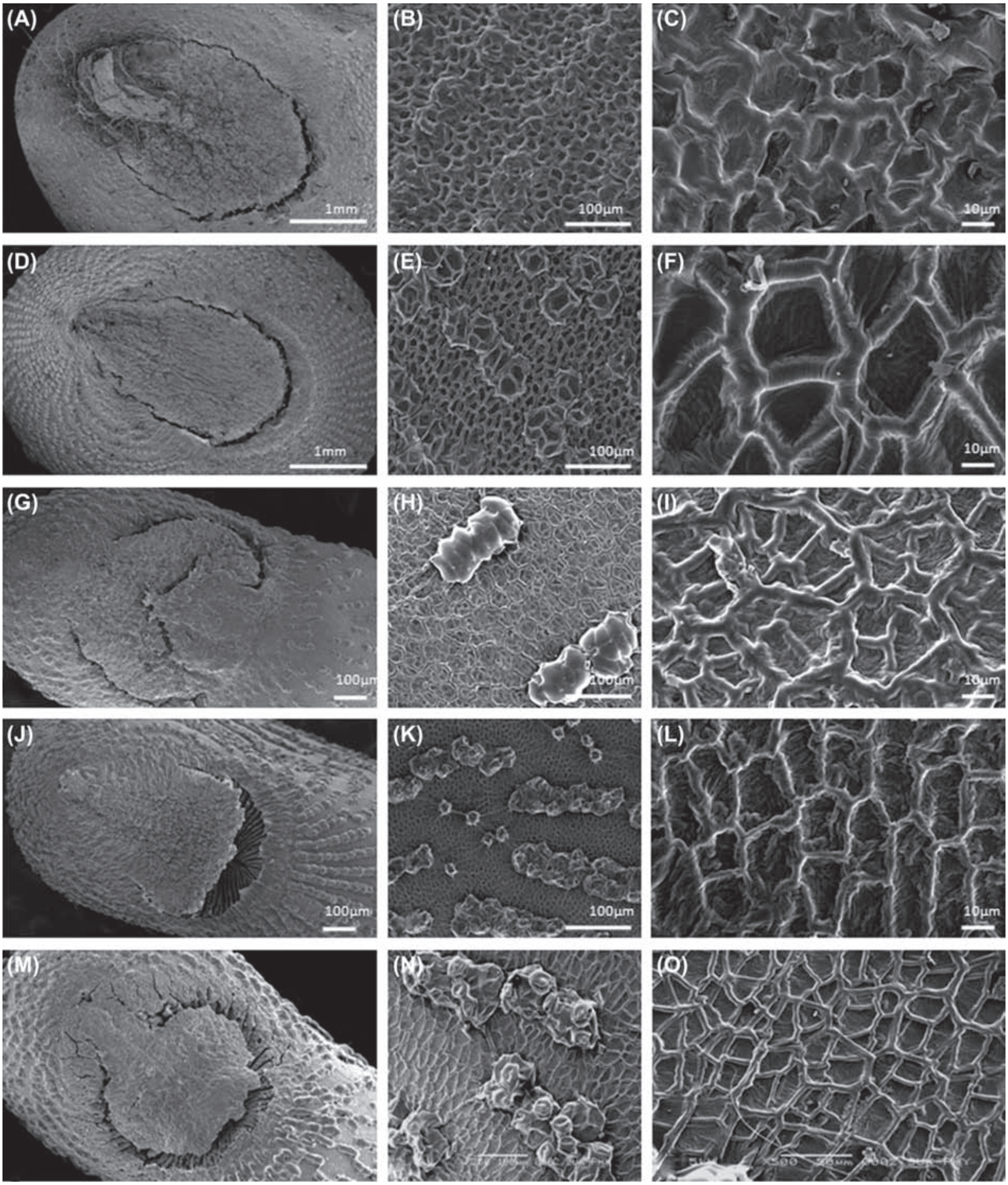

Figure 3. SEM micrographs of hilum, surface topography and seed coat pattern of seeds of *Abelmoschus* species. (A)–(C) *A. esculentus*, (D)–(F) *A. caillei*, (G)–(I) *A. crinitus*, (J)–(L) *A. moschatus* subsp. *moschatus*, (M)–(O) *A. moschatus* subsp. *tuberosus*.

that seed macro- and micro-morphological characters are of potential taxonomic importance for the identification of many species of *Abelmoschus*.

The current taxonomic consensus is in favor of treating *Abelmoschus* as a distinct genus distinguished from *Hibiscus* by its characteristic asymmetrical, spathaceous, deciduous calyx, as opposed to the campanulate or copular, regular, accrescent calyx of *Hibiscus* (Sivarajan and Pradeep 1996). Moreover, Mwachala (1995) reported trichomes on the seed surface of some species of *Hibiscus* sect. *Furcaria* to be comprised of a scale like structure formed by fused unicellular hairs. In contrast, seed trichomes (where present) in *Abelmoschus* were by us found to be unicellular, unfused, spiral or non-spiral structures. Our results thus indicate that seed micro-morphology, especially trichome structure, may play a decisive role in the generic separation of *Abelmoschus* from *Hibiscus*.

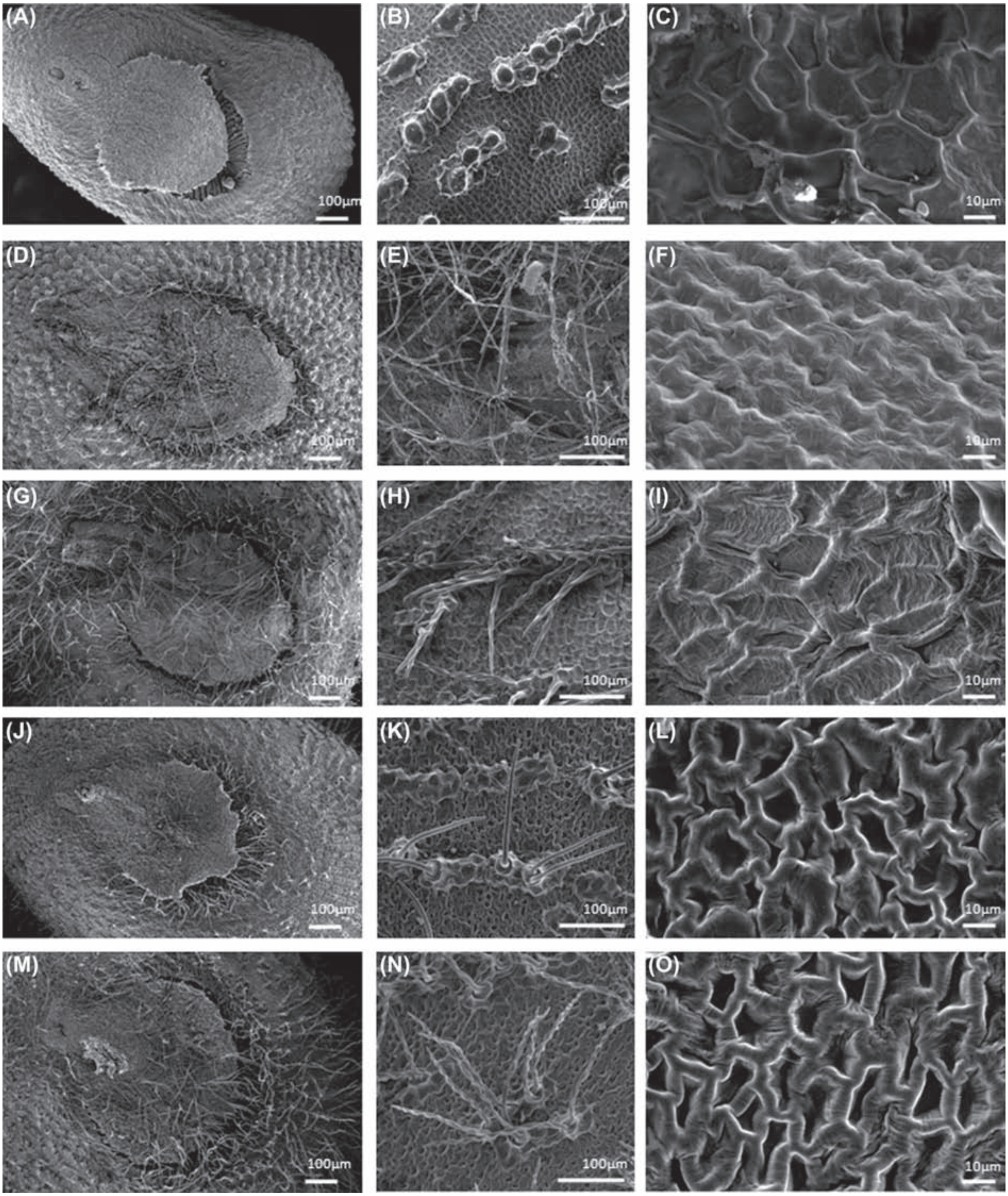

Figure 4. SEM micrographs of hilum, surface topography and seed coat pattern of *Abelmoschus* species. (A)–(C) *A. enbeepeegearense*, (D)–(F) *A. ficulneus*, (G)–(I) *A. tuberculatus*, (J)–(L) *A. manihot* subsp. *manihot*, (M)–(O) *A. manihot* subsp. *tetraphyllus* var. *tetraphyllus.*

Seed dimensions shows great variation among *Abelmoschus* species, with the cultivated taxa (*A. esculentus* and *A. caillei*) having almost similar seed size (Table 2). Among the cultivated species, two species, i.e. *A. esculentus* (Asian genotypes) and *A. caillei* (west African genotypes) show great similarities in reproductive features and are generally difficult to identify correctly. However, the results from our seed micro-morphological study revealed impressive differences between *A. esculentus* and *A. caillei*, thus further confirming the findings of Martin and Rhodes (1983), Stevels (1988) and Sunday et al. (2008).

A simple-reticulate testa pattern is relatively consistent among the studied species of *Abelmoschus*. However, three species showed unique and species-specific testa patterns: reticulate-storied (*A. crinitus*, Fig. 3I and *A. moschatus* subsp. *tuberosus*, Fig. 3O) and reticulate-foveate (*A. ficulneus*, Fig. 4F) that differentiate them from all other taxa studied. *Abelmoschus crinitus* also has the unique feature of smooth

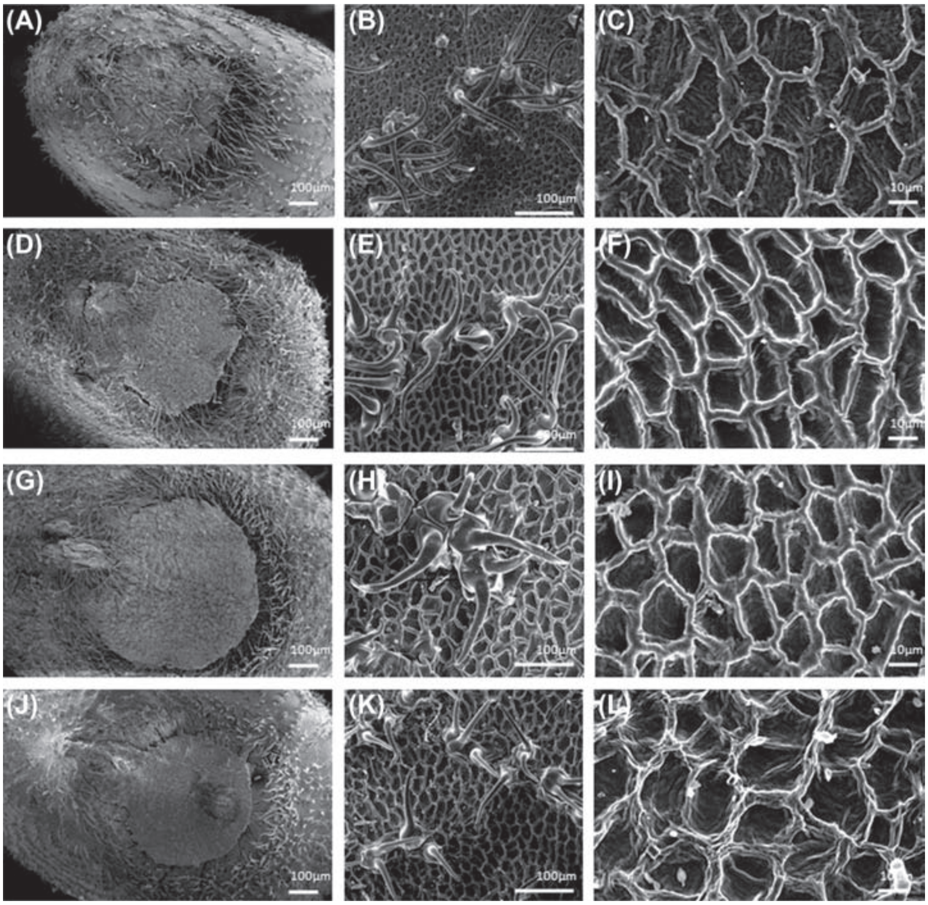

Figure 5. SEM micrographs of hilum, surface topography and seed coat pattern of *Abelmoschus* species. (A)–(C) *A. manihot* subsp. *tetraphyllus* var. *pungens*, (D)–(F) *A. angulosus* var. *grandiflorus*, (G)–(I) *A. angulosus* var. *purpureus*, (J)–(L) *A. angulosus* var. *angulosus.*

protuberances in a row pattern on the seed surface, while *A. moschatus* subsp. *tuberosus* has remnants of trichomes distinguishing these two species from each other. Our results also indicate that these character states are stable across the habitat range of these two species.

Abelmoschus moschatus has been considered as a polymorphic species by many botanists. i.e. Masters (1874),

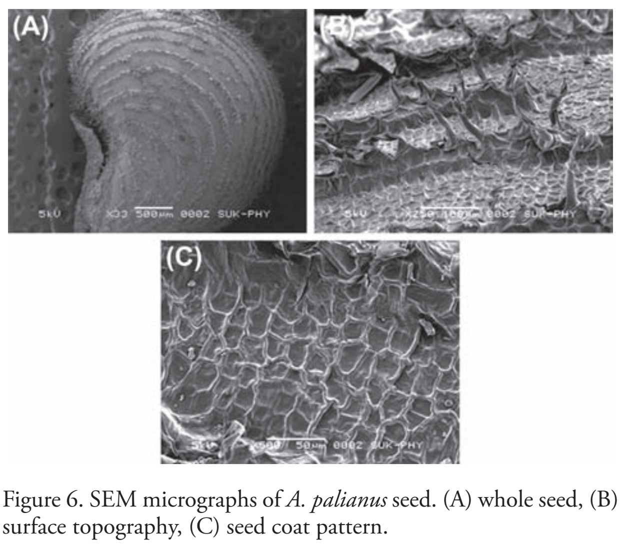

Figure 6. SEM micrographs of *A. palianus* seed. (A) whole seed, (B) surface topography, (C) seed coat pattern.

Hochreutiner (1900) and Van Borssum-Waalkes (1966), and therefore they recognized many subspecies and varieties. *Abelmoschus* subsp. *moschatus* and *A. moschatus* subsp. *tuberosus* are clearly part of a species complex. Some common exclusive features, especially seed shape and remnants of trichomes in concentric rows on the seeds were observed only in *A. moschatus* subsp. *moschatus* and *A. moschatus* subsp. *tuberosus*. Further, our investigations revealed a remarkable variability in seed coat pattern within this species, i.e. a storied reticulate pattern in *A. moschatus* subsp. *tuberosus* but simple reticulate in *A. moschatus* subsp. *moschatus*. These results gives further support to Bates (1968) who proposed to elevate *A. moschatus* subsp. *tuberosus* to specific rank. Taken the above fact into consideration, we suggest that *A. moschatus* subsp. *tuberosus* should be elevated to a separate species since it is morphologically separable and clearly distant from *A. moschatus* subsp. *moschatus*.

Another interesting new entity is *A. enbeepeegearense*, recently identified by John et al. (2012) from the southern Western Ghats, showing characters intermediate between the closely related species *A. moschatus* subsp. *tuberosus* and *A. crinitus*. In the present study, *A. enbeepeegearense* was found to be similar to *A. moschatus* subsp. *tuberosus* with respect to seed shape (Fig. 2F) and to *A. crinitus* with respect to the seed color (Fig. 1F), thus partially supporting John et al. (2012). However, the possibility of *A. enbeepeegearense* being a natural hybrid between *A. moschatus* subsp. *tuberosus* and *A. crinitus* should not be neglected, since considerable amount of natural cross-pollination (4–19%, Shalaby 1972) with a maximum of 42.2% (Mitidieri and Vencovsky 1974) has been reported in *Abelmoschus*.

Species of *Abelmoschus* exhibit several highly variable leaf, flower and fruit characters that have been used extensively in previous classifications (Van Borssum-Waalkes 1966, Sivarajan and Pradeep 1996, Osawaru et al. 2011, John et al. 2012, Sutar et al. 2013). In contrast, trichome structure and type has received less attention than flowers and fruits as potentially useful character for delimiting species. Prantl (1891) is the only taxonomist who proposed the use of trichome type to segregate Cruciferae taxa at the tribal level. Trichome distribution on the seeds shows obvious variation within *Abelmoschus*, and is easily observable using light microscopy. Besides the vital importance of trichomes in *Abelmoschus* taxonomy, their evolutionary and ecological aspects should not be overlooked. In fact, there seems to be a strong correlation between the type of habitat and trichome distribution in a species. The presence of trichomes on the seeds of *A. tuberculatus* and its occurrence along roadsides and grassy slopes may support Van Borssum-Waalkes (1966) who considered it as a wild progenitor of *A. esculentus*. However, critical observations on seed surface revealed the distinctness of *A. tuberculatus* from *A. esculentus* and therefore supports the recognition of *A. tuberculatus* as a distinct species as proposd by Pal et al. (1952).

Very few attempts have been made to trace the evolutionary history of cultivated okra. However, phylogenetic information could be useful in okra improvement programs. Recently, a nrDNA and cpDNA based phylogenetic analysis by Ramya and Bhat (2012) did not support the evolutionary background of *A. esculentus* proposed earlier (Joshi and Hardas 1956). In the present study, among the probable second parents of *A. esculentus*, *A. moschatus* was found to be highly distinct from *A. esculentus* in seed shape (though both have the deciduous type of trichomes). This species is also renowed for its extensive pre-fertilization barriers, when crossed with *A. esculentus* (Patil et al. 2013). Hence, the present information is in favor of *A. ficulneus* as the most probable second parent of *A. esculentus*, rather than *A. moschatus*, as both these species exhibited a tightly joined, polygonal epidermal cell structure (Table 3).

For the perennial taxon *A. manihot* subsp. *tetraphyllus* var. *pungens*, the present findings contradict the conclusion of Hochreutiner (1900), Van Borssum-Waalkes (1966), Paul and Nayar (1988) and Paul (1993) who established it as a variety of *A. manihot* subsp. *tetraphyllus*. Our observation on seed size, seed shape, trichome structure and anticlinal wall structure of seeds of this taxon instead shows close similarity to those of *A. angulosus*. However, in contrast, Vredebregt (1991) demonstrated that *A. manihot* subsp. *tetraphyllus* var. *pungens* is not much different from var. *tetraphyllus*. However, in the present study *A. manihot* subsp. *tetraphyllus* var. *pungens* was the only taxon with presence of a triangular shaped hilum and highly wrinkled periclinal walls, while *A. manihot* subsp. *tetraphyllus* var. *tetraphyllus* was found to be characterized by a rounded shaped hilum and smooth periclinal walls. Thus, hilum shape and periclinal wall structure may play a significant role in differentiating these two taxa from each other. Moreover, the present facts reinforce the need of a taxonomic revision of these taxa based on a multidisciplinary approach.

Hemon et al. (1987) pointed out that *A. manihot* subsp. *manihot* and *A. manihot* subsp. *tetraphyllus* are difficult to keep separate since general morphology does not provide diagnostic characters. Due to the inconsistency and fragility of the characters used by Van Borssum-Waalkes (1966) Paul and Nayar (1988) and Paul (1993), Sivarajan and Pradeep (1996) did not consider their infra-specific classification of *A. manihot* and preferred to treat *A. manihot* as a single species without infraspecific taxa. In the present study, our LM and SEM observations on multiple samples revealed that trichome density and orientation are diagnostic for these two taxa: sparsely distributed and non-spiral trichomes in *A. manihot* subsp. *manihot* but densely distributed, spiral trichomes in *A. manihot* subsp. *tetraphyllus*. However, both taxa had nearly the same seed coat pattern under high magnification, an observation which may partially support the conclusion of Sivarajan and Pradeep (1996). Apart from morphological variability, this species showed great distinctness in seed micro-morphological characters stressing the need to study *A. manihot* subsp. *manihot*, *A. manihot* subsp. *tetraphyllus* var. *tetraphyllus*, and var. *pungens* with advanced molecular markers (Ramya and Bhat 2012, Schafleitner et al. 2013).

The obtained results confirm the usefulness of seed morphology for the identification of infraspecific taxa in *A. angulosus*. Based on variation in flower color, Sivarajan and Pradeep (1996) proposed three varieties, namely *A. angulosus* var. *grandiflorus* (yellow corolla), *A. angulosus* var. *angulosus* (white corolla) and *A. angulosus* var. *purpureus* (pink corolla). The present study adds two important seed characters which are able to differentiate between these varieties: *A. angulosus* var. *grandiflorus* (epidermal cell tetragonal or pentagonal, elongate; anticlinal wall smooth), *A. angulosus* var. *angulosus* (epidermal cell polygonal; anticlinal wall thick, wrinkled) and *A. angulosus* var. *purpureus* (epidermal cell tetragonal or pentagonal, not elongate; anticlinal wall thick, smooth) and thereby supports the treatments of Thwaites (1858) and Sivarajan and Pradeep (1996). The present study also supports Sutar et al. (2013), who described *A. palianus* as a distinct species. This species is distinct from *A. angulosus* by having non-deflected (i.e. stiffed), unicellular trichomes while *A. angulosus* has deflected and di–tri cellular trichomes.

Some intra-specific variation were also observed (data not shown) in the seed macro- and micro-morphological characters of the studied *Abelmoschus* species. However, all the species, except the cultivated *A. esculentus*, showed very little variability (not significant) for characters like seed color, seed size, and seed coat pattern. Intra-specific variation of seed coat surface in the cultivated genotypes of *A. esculentus* may have been influenced by genetic and environmental interactions due to their different ecological habitats (Wyatt 1984) and selection during cultivation, and is therefore fully concordant with Sunday et al. (2008).

To conclude, the current study provides a basic, detailed and comprehensive seed morphological comparison among

Abelmoschus species, and reveal novel characters to be considered in the taxonomy of the genus. Based on the seed coat pattern, we validate the distinctness of *A. manihot* subsp. *tetraphyllus* var. *pungens* from *A. manihot* subsp. *tetraphyllus* var. *tetraphyllus*, and the cultivated *A. esculentus* from *A. caillei*. Furthermore, our results stresses the need of undertaking a thorough study of *Abelmoschus* taxonomy and evolution, which may form the basis for the identification and scientific management of the species (both cultivated and wild) of *Abelmoschus* in future research, breeding and conservation efforts.

Acknowledgements – This work was conducted under sub-project 'Biosystematics study of the genera *Vigna*, *Cucumis* and *Abelmoschus*' funded by National Agriculture Innovation Project (NAIP) of Indian Council of Agriculture Research (ICAR), Government of India. The facilitation of work by Dr K. C. Bansal, Director, NBPGR, New Delhi is acknowledged.

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