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RESEARCH ARTICLE

Integrating morphology with fruit and seed micromorphology for the taxonomic delimitation of selected species of the subtribe Cassiinae (Fabaceae) in Assam, India

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Abstract

Integrative morphological analyses combining macroscopic measurements with scanning electron microscopy (SEM) provide important taxonomic evidence for delimiting closely related legume taxa within Cassiinae (Fabaceae). This study examines the micromorphological diversity of fruits and seeds in six species of the subtribe Cassiinae from Assam, located within the Indo–Burma biodiversity hotspot: *Cassia javanica* L., *Senna surattensis* (Burm.f.) H.S. Irwin & Barneby, *Senna occidentalis* (L.) Link, *Senna tora* (L.) Roxb., *Senna hirsuta* (L.) H.S. Irwin & Barneby and *Senna sophera* (L.) Roxb.. Mature fruits and seeds were sampled across multiple habitats in Assam during peak fruiting and key metrics (fruit length, width, seed length, width, 100-seed mass and L/B ratios) were recorded. Scanning electron microscopy examination was used to study seed coat micromorphology, focusing on spermoderm ornamentation, cuticle integrity, wax deposition and cell wall projections. Multiple morphometric and qualitative SEM characters were evaluated for their diagnostic utility in six species. Distinct fruit dehiscence types (indehiscent vs dehiscent), seed-coat patterns (tuberculate, rugose, reticulate) and L/W ratios emerged as reliable taxonomic markers. *Cassia javanica* was characterised by large, indehiscent pods and seeds with a thick, waxy testa, whereas *S. occidentalis* and *S. sophera* showed comparatively thinner, tuberculate seed coats. The combined macro and micromorphological evidence supported species-level delimitation and was broadly consistent with Irwin and Barneby's taxonomic framework for Cassiinae. These findings highlight the taxonomic significance of fruit and seed micromorphology in Cassiinae. By providing integrative diagnostic characters and aligning them with established taxonomic frameworks, this study strengthens species delimitation and identification of Cassiinae (Fabaceae) taxa from Assam.

Keywords: Assam; Cassiinae; Fabaceae; legume taxonomy; scanning electron microscopy; seed coat micromorphology

Introduction

The legume subtribe Cassiinae (Fabaceae: Caesalpinioideae) comprises the genera *Cassia* L. and *Senna Mill.*, with *ca.* 330 species (37 in *Cassia* and 293 in *Senna*) distributed as shrubs, trees and herbs across tropical and subtropical regions worldwide (1). Within this diverse lineage, Asia supports a diverse assemblage of *Senna* species adapted to varied ecological niches. Northeast India, part of the Indo –Burma biodiversity hotspot, is especially rich in endemic and economically significant plant taxa (2). The Ministry of Environment and Forests (MoEF), Government of India, recorded 8 species of Cassiinae from Assam, used by various tribes of Assam in the treatment of about 40 different diseases (3). Despite this diversity, morphological and micromorphological studies of fruit and seed characters in Cassiinae taxa in India remain sparse, limiting taxonomic resolution and hindering accurate species delimitations.

Fruit and seed morphology have emerged as valuable tools for resolving taxonomic ambiguities within the subtribe Cassiinae (Fabaceae). Characters such as fruit shape, size, surface texture, seed dimensions and external seed coat ornamentation are useful taxonomic markers, primarily because of their morphological stability and relatively stable morphological characters under varying environmental conditions during seed maturation (4–6). Such stability ensures consistent morphological patterns that significantly contribute to systematic and phylogenetic classifications.

Fruit morphology including pod dehiscence, septation and pod shape has long been recognised as taxonomically important in subtribe Cassiinae (7–9). Similarly, seed-coat micromorphology revealed by scanning electron microscopy (SEM) provides high-resolution micromorphological characters, such as spermoderm patterns, cuticular features and epicuticular wax deposits, that can distinguish cryptic taxa and resolve phylogenetic relationships (10–13). Scanning electron microscopy studies have also provided reliable taxonomic criteria by highlighting distinct micromorphological features, such as seed-coat ornamentation patterns, hilum morphology, testa architecture and micropyle details, which are valuable for accurate interspecific and intergeneric delineation (14–19). Modern integrative taxonomy increasingly relies on such combined macro- and micromorphological evidence to refine species delimitations and validate sectional classifications (20).

This study focuses on six taxa of the genera *Senna* and *Cassia* that represent the principal fruit and seed morphotypes encountered in ecosystems of Assam and hold significant ethnobotanical value. *Cassia javanica* L., *Senna surattensis* (Burm.f.) H.S. Irwin & Barneby, *Senna occidentalis* (L.) Link, *Senna tora* (L.) Roxb., *Senna hirsuta* (L.) H.S. Irwin & Barneby and *Senna sophera* (L.) Roxb. (Fig. 1–6). These taxa occur within tropical to seasonally dry tropical ecological zones in Assam and the wider Indian subcontinent and selected members of *Senna* and *Cassia* are documented as medicinally or environmentally useful taxa in authoritative plant use databases, floristic inventories and regional ethnobotanical studies from Assam (21–22). Based on the foundational revision from a previous study (23), our objectives are to (1) quantify key fruit and seed dimensions and 100-seed weight; (2) characterize seed-coat ornamentation and micromorphological features via SEM; (3) evaluate the diagnostic and systematic utility of combined macro- and micromorphological characters; and (4) develop an updated identification key for *Cassia* and *Senna* species of Assam. In this study, the authors used high-resolution images to enhance taxonomic clarity and support conservation of Assam's endemic legume flora. It also provides a morphological framework for future integrative phylogenetic studies.

Materials and Methods

Mature, dry fruits and seeds of six selected species from these two genera, *Senna* and *Cassia*, were randomly collected during the peak fruiting season from natural habitats in Assam. Each specimen was labelled and stored in clean, dry paper envelopes to prevent deterioration.

Macromorphological study

For each species, fruit length and width were measured using a digital vernier caliper. Pod and seed images were calibrated in ImageJ 1.54 g to insert scale bars in the macromorphological figure panels. The number of seeds per fruit was recorded as the mean value from 10 mature fruits per species. The type of fruit dehiscence was noted and the fruiting period was documented during each field visit and in herbarium records.

Twenty seeds per species were examined for colour, general shape (e.g., ovate, oblong), surface texture (e.g., reticulate, smooth, tuberculate) and hilum length and width. Observations were recorded using a stereomicroscope and hand lenses, when required.

Micromorphological study

Seed coat features were examined using SEM at magnifications of 62X, 500X, 1000X and 4000X. Samples were gold-coated using a sputter coater before imaging. Scanning electron microscopy observations were used to categorise spermoderm patterns and explore micromorphological features including cuticle integrity, wax deposition and cell wall projections.

Seed weight and the seed length-to-width ratio

To evaluate seed mass, 100 seeds per species were weighed using a digital microbalance. The length-to-width (L/W) ratio was calculated to describe seed shape variation among the studied species.

Statistical analysis

Multivariate morphometric analysis

Principal component analysis (PCA) and hierarchical clustering were performed using the PAST software version 4.03. The PCA was based on six quantitative morphological variables: fruit length (cm), fruit width (cm), fruit length-to-width ratio (Fruit L/W), seed length (mm), seed width (mm) and seed length-to-width ratio (Seed L/W). All variables were standardised before analysis to minimise the effect of differences in measurement scale.

Hierarchical cluster analysis was conducted using Ward's minimum variance method with Euclidean distance as the distance measure. The goodness of fit of the dendrogram was assessed using the cophenetic correlation coefficient (CCC), calculated as the correlation between the original Euclidean distance matrix and the cophenetic distance matrix derived from the clustering.

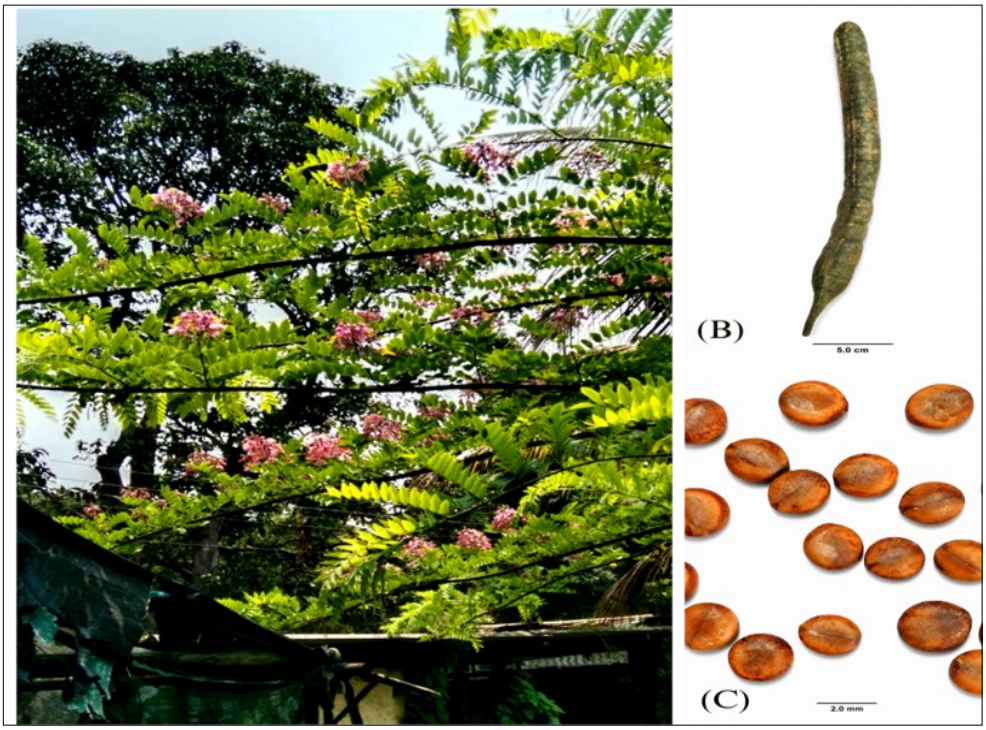


Fig. 1. Macromorphological features of *Cassia javanica*. A: Plant; B: Pod; C: Seeds.

Scale bars: B = 5.0 cm; C = 2.0 mm.

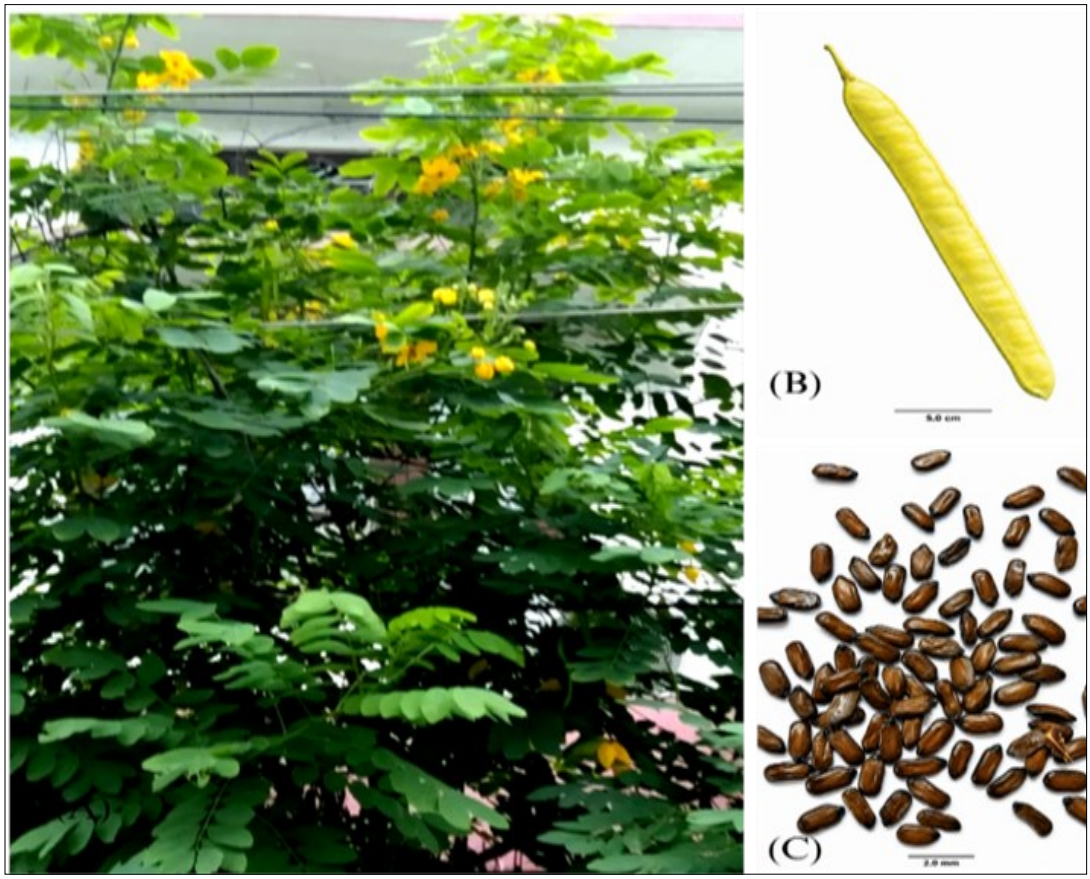


Fig. 2. Macromorphological features of *Senna surattensis*. A: Plant; B: Pod; C: Seeds.
Scale bars: B = 5.0 cm; C = 2.0 mm.

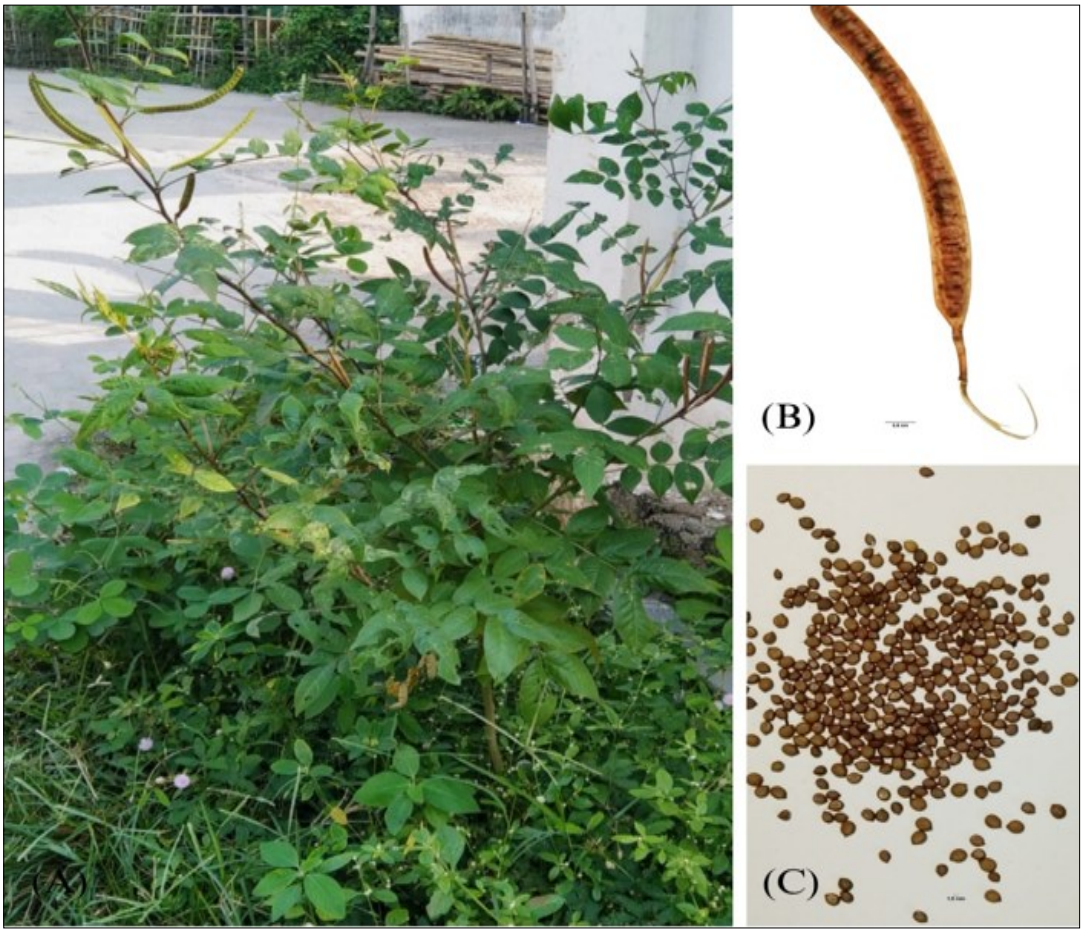


Fig. 3. Macromorphological features of *Senna occidentalis*. A: Plant; B: Pod; C: Seeds.
Scale bars: B = 3.0 cm; C = 1.0 mm.



Fig. 4. Macromorphological features of *Senna tora*. A: Plant; B: Pod; C: Seeds.
Scale bars: B = 3.0 cm; C = 1.0 mm.



Fig. 5. Macromorphological features of *Senna hirsuta*. A: Plant; B: Pod; C: Seeds.
Scale bars: B = 3.0 cm; C = 0.5 mm.



Fig. 6. Macromorphological features of *Senna sophera*. A: Plant; B: Pod; C: Seeds.

Scale bars: B = 3.0 cm; C = 1.0 mm.

Results

Examination of fruit morphology across the six studied taxa indicated considerable interspecific variation in pod dehiscence, dimensions and external features (Table 1). *Cassia javanica* was characterised by indehiscent, straight, cylindrical pods measuring 27.0–27.1 cm in length and 1.69–1.71 cm in width, with a distinctive transverse one-seeded compartment and a glabrous, terete surface. In contrast, the studied *Senna* species (*S. sophera*, *S. surattensis*, *S. tora, S. occidentalis and S. hirsuta)* exhibited dehiscent pods with varying morphologies. *Senna surattensis* produced flat, strap-shaped pods (19.6–19.8 cm long). In contrast, *S. hirsuta* exhibited pods that are subterete and curved, with a conspicuous strigose indumentum.

Pod slenderness, expressed as length-to-width ratios, varied considerably among species. *Senna hirsuta* exhibited the slenderest pods (L/W ratio = 28.5–29.5), followed by *S. tora* (L/W ratio = 20.0–21.0), while *C. javanica* showed the lowest ratio (15.90). Seed production per fruit ranged from 15–17 seeds in *S. surattensis* to 78–82 in *S. hirsuta*, with *C. javanica* producing an intermediate range of 23–25 seeds per pod.

Seed macromorphology

Seed morphological analysis showed a unique interspecific pattern in shape, size and mass characteristics (Table 2). Seed shapes varied among species considerably: *C. javanica* and *S. sophera* produced ovate seeds, *S. surattensis* had oblong seeds, *S. tora* exhibited rhomboidal seeds and both *S. occidentalis* and *S. hirsuta* had obovate seeds. Seed dimensions showed marked variation, with seed lengths ranging from 2.0–2.1 mm in *S. hirsuta* to 7.9–8.1 mm in *S. surattensis* and seed width ranging from 1.0–1.1 mm (*S. hirsuta*) to 5.9–6.1 mm (*C. javanica*).

The 100-seed mass varied by approximately 20-fold among species, from 59–60 mg in *S. hirsuta* to 1130–1140 mg in *C. javanica*. Seed length-to-width ratios also reflected shape variation, with *S. surattensis* showing the highest ratio (2.6–2.7). *Senna surattensis* displayed highly elongated seeds. On the other hand, *C. javanica* had the lowest ratio (1.1–1.2), reflecting broader, more rounded seeds.

Seed coat micromorphology

Scanning electron microscopy showed spermoderm ornamentation patterns that correlated with taxonomic groupings (Fig. 7–12). Two primary surface patterns were identified: reticulate and tuberculate. *Cassia javanica* and *S. hirsuta* exhibited reticulate ornamentation characterised by elongated cells with heavy wax deposition and thick, cracked cuticles. *Senna surattensis* displayed a unique glossy, regular surface with smooth, flat cells. In contrast, *S. occidentalis*, *S. tora* and *S. sophera* showed tuberculate patterns with regular, compact protuberances covering the seed surface.

High-magnification SEM imaging (4000X) revealed additional details of seed coat micromorphology. *Cassia javanica* seeds showed thick, heavily fissured cuticles with large rugose projections, potentially serving protective functions. *Senna hirsuta* seeds displayed loosely arranged rugose structures with thin cuticles, while the tuberculate species (*S. occidentalis*, *S. sophera* and *S. tora*) exhibited tightly packed, regular tubercles of varying sizes.

Multivariate morphometric analysis

The PCA showed that the first three components accounted for 92.1 % of the total variation (Fig. 13). Principal component 1 (PC1) (63.4 % of variance) described a "size and mass axis" dominated by fruit and seed dimensions. Whereas, PC2 (16.5 % of variance) reflected characteristics of seed shape and proportion. *Cassia javanica* showed a positive PC1 score (4.69) in its large fruit and seed

Table 1. Fruit morphology and seed number of selected Cassiinae taxa from Assam

Sl. No.	Name of taxa	Fruit type and characteristics	Length of fruit (L) (cm)	Width of fruit (W) (cm)	Ratio of length and width (R = L/W)	Total no. of seeds per fruit
1	*C. javanica*	Pod straight, cylindrical, terete and glabrous; internally divided by transverse septa into one-seeded compartments; indehiscent, black	27.0–27.1	1.69–1.71	15.5–16.0	23–25
2	*S. surattensis*	Flat pod, strap-shaped, dehiscent, thickened on sutures, pubescent, depressed between the seeds, dark brown	19.6–19.8	1.29–1.31	14.8–15.5	15–17
3	*S. occidentalis*	Flat pod, slightly curved, compressed, transversely septate, dehiscent, brown	13.1–13.3	0.89–0.91	14.4–14.9	39–41
4	*S. tora*	Linear-cylindric pod, straight or slightly curved, dehiscent, obliquely septate, narrow at the base, black	12.2–12.4	0.59–0.61	20.0–21.0	17–19
5	*S. hirsuta*	Linear pod, subterete, curved outward, dehiscent, sinuate, minutely strigose, transversely septate, brownish-white	14.4–14.6	0.49–0.51	28.5–29.5	78–82
6	*S. sophera*	Linear-cylindric pod, straight or slightly curved, dehiscent, obliquely septate, narrow at the base, brown	11.3–11.5	0.79–0.81	13.9–14.6	49–51

L: length; W: width; R: length/width ratio.

Table 2. Seed morphology, scanning electron microscopy (SEM) seed-surface characteristics and germination of selected Cassiinae taxa from Assam

Sl. No.	Taxa	Seed colour	Seed shape	Seed surface (SEM)	Length (mm)	Width (mm)	L/W Ratio	100-Seed weight (mg)	Pre-germination treatment	Germination (%)	Germination time (hr)
1	*C. javanica*	Glossy light brown	Ovate, flat with shallow longitudinal depression	Spermoderm reticulate type with heavy wax deposition; thick, cracked cuticle	6.9–7.1	5.9–6.1	1.1–1.2	1130–1140	Hot water treatment followed by soaking	60	58
2	*S. surattensis*	Dark brown	Oblong, flat, truncated at ends	Spermoderm regular to smooth type with glossy surface	7.9–8.1	2.9–3.1	2.6–2.7	327–329	Hot water treatment followed by soaking	95	50
3	*S. occidentalis*	Pale brown	Obovate to suborbicular, flattened	Spermoderm tuberculate, regular and compactly arranged	4.9–5.1	3.9–4.1	1.2–1.3	188–190	Water soaking only	87	24
4	*S. tora*	Greyish brown	Rhomboidal, cylindrical with oblique ends	Spermoderm tuberculate, confined to the pleurogram region	2.9–3.1	1.9–2.1	1.4–1.6	116–118	Water soaking only	93	36
5	*S. hirsuta*	Dark brown	Obovate with hilum line on both faces	Spermoderm reticulate to rugose, loosely arranged; thin cuticle	2.0–2.1	1.0–1.1	1.9–2.1	59–60	Water soaking only	98	30
6	*S. sophera*	Pale brown	Obovate to suborbicular, flattened	Spermoderm tuberculate, regular and compactly arranged	3.9–4.1	2.9–3.1	1.3–1.4	186–188	Water soaking only	90	48

L/W: length-to-width ratio; SEM: scanning electron microscopy.

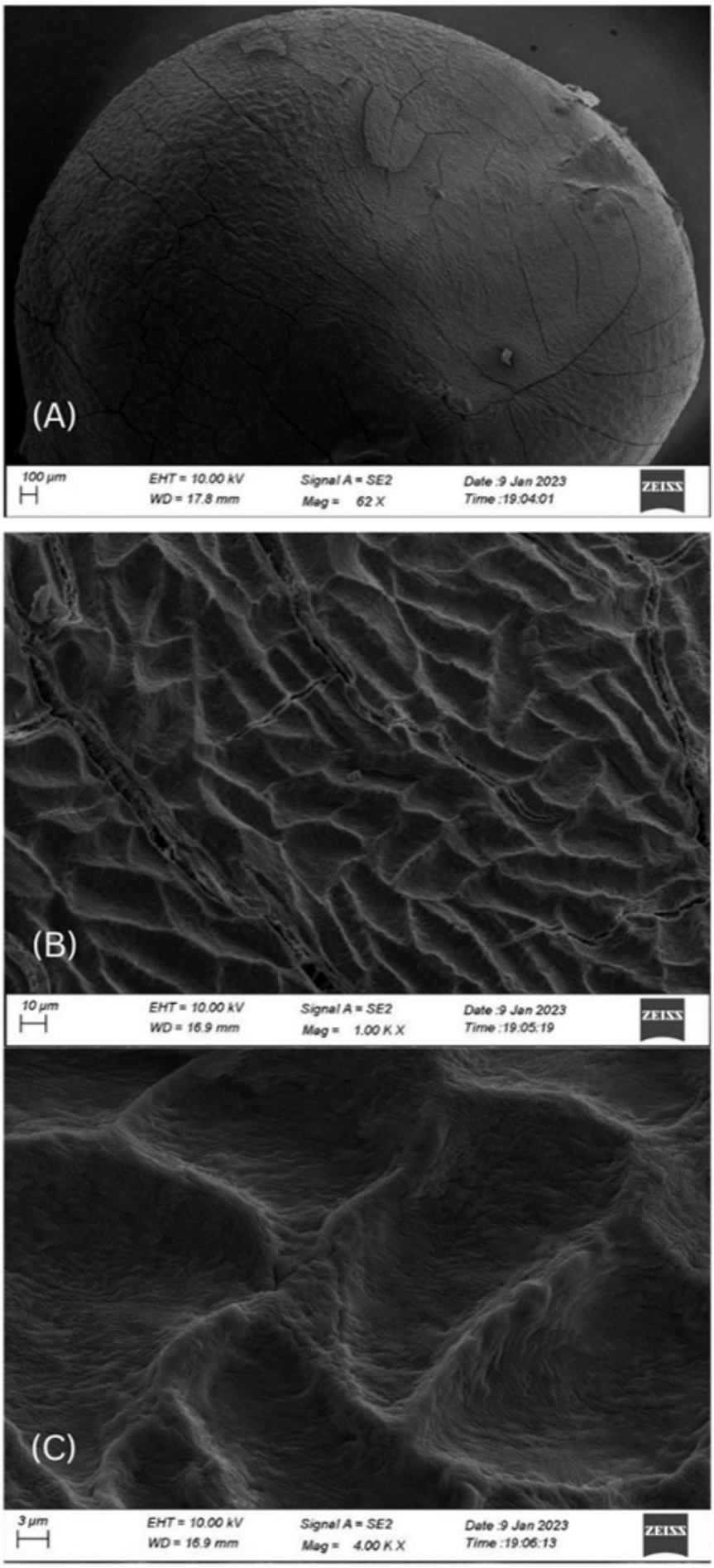


Fig. 7. Scanning electron microscopy (SEM) micrographs of seed morphology and ornamentation of *Cassia javanica*. A: Magnification: 62X, Scale bar: 100 µm; B: Magnification: 1000X, Scale bar: 10 µm; C: Magnification: 4000X, Scale bar: 3 µm

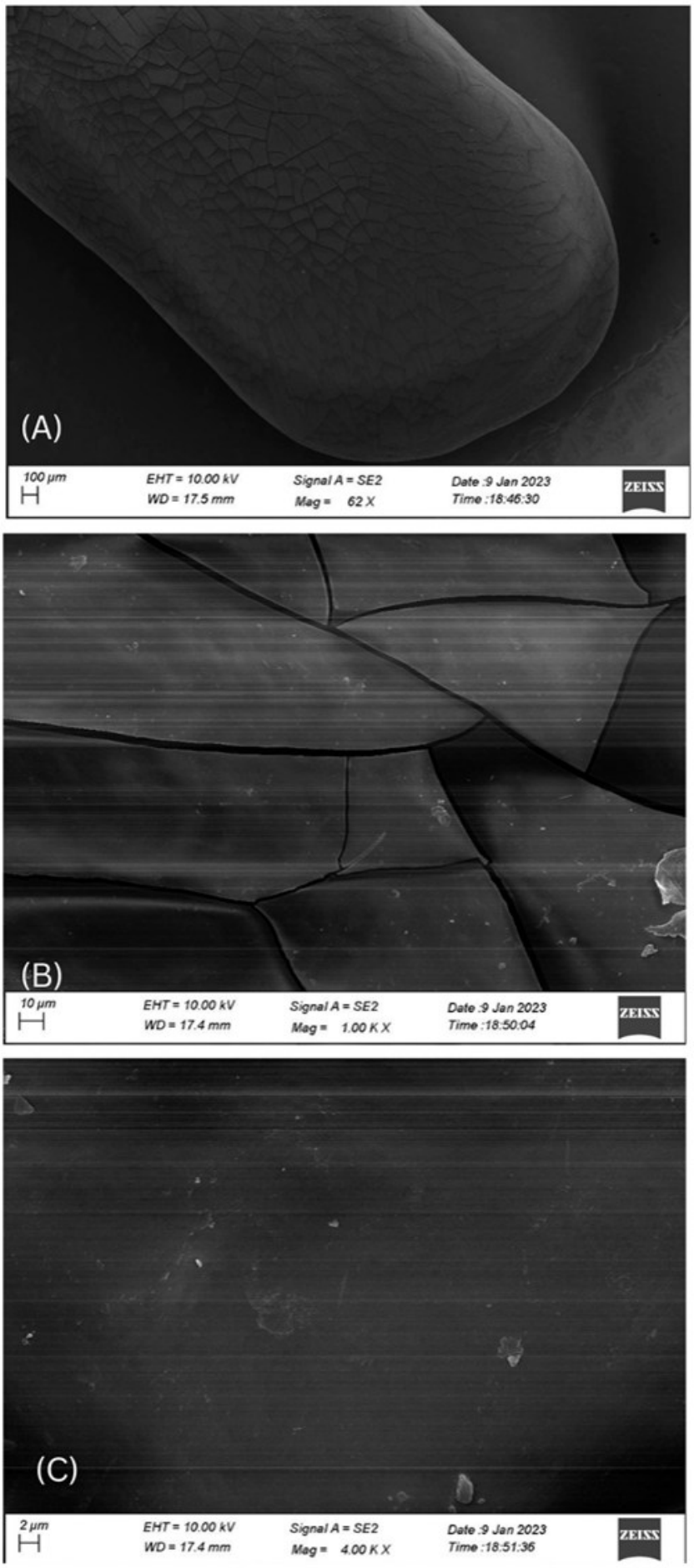


Fig. 8. Scanning electron microscopy (SEM) micrographs of seed morphology and ornamentation of *Senna surattensis*. A: Magnification: 62X, Scale bar: 100 µm; B: Magnification: 1000X, Scale bar: 10 µm; C: Magnification: 4000X, Scale bar: 3 µm.

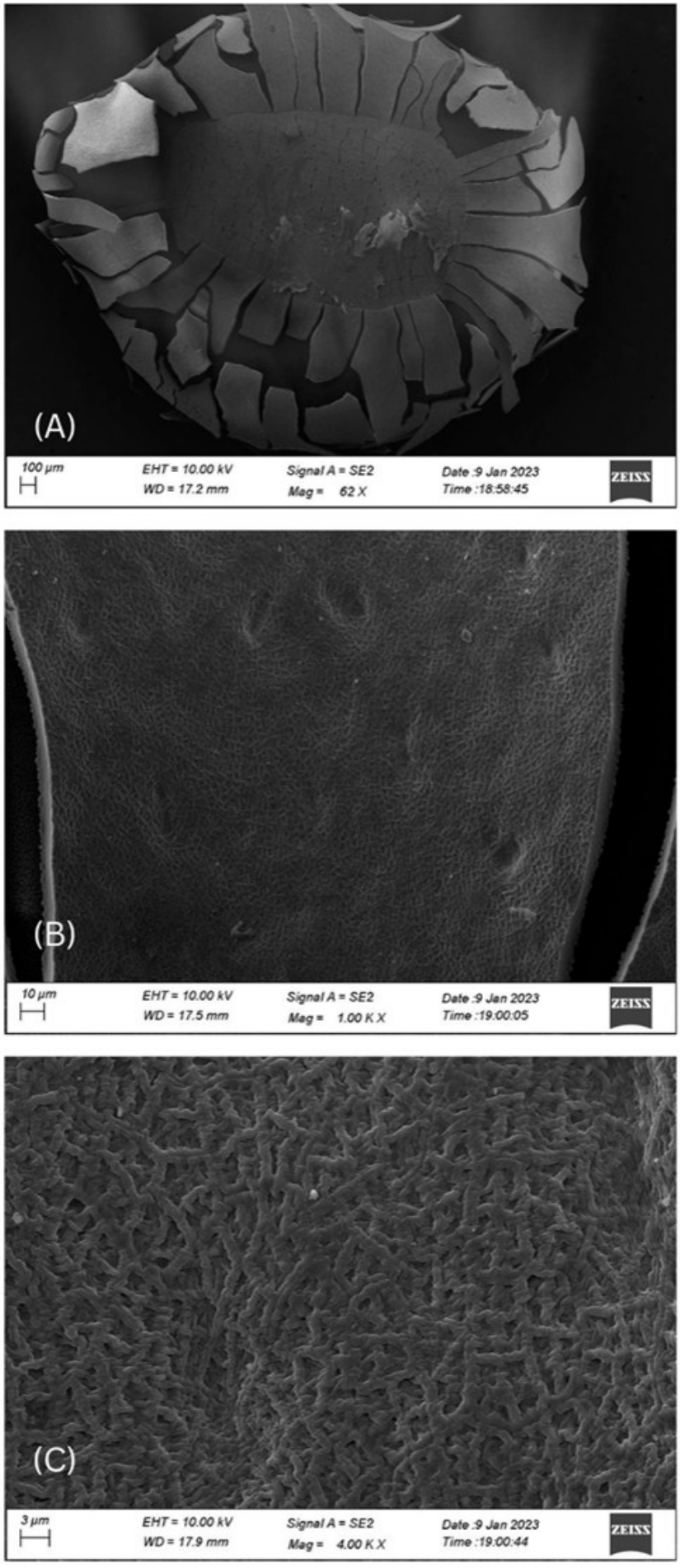


Fig. 9. Scanning electron microscopy (SEM) micrographs of seed morphology and ornamentation of *Senna occidentalis*. A: Magnification: 62X, Scale bar: 100 µm; B: Magnification: 1000X, Scale bar: 10 µm; C: Magnification: 4000X, Scale bar: 3 µm.

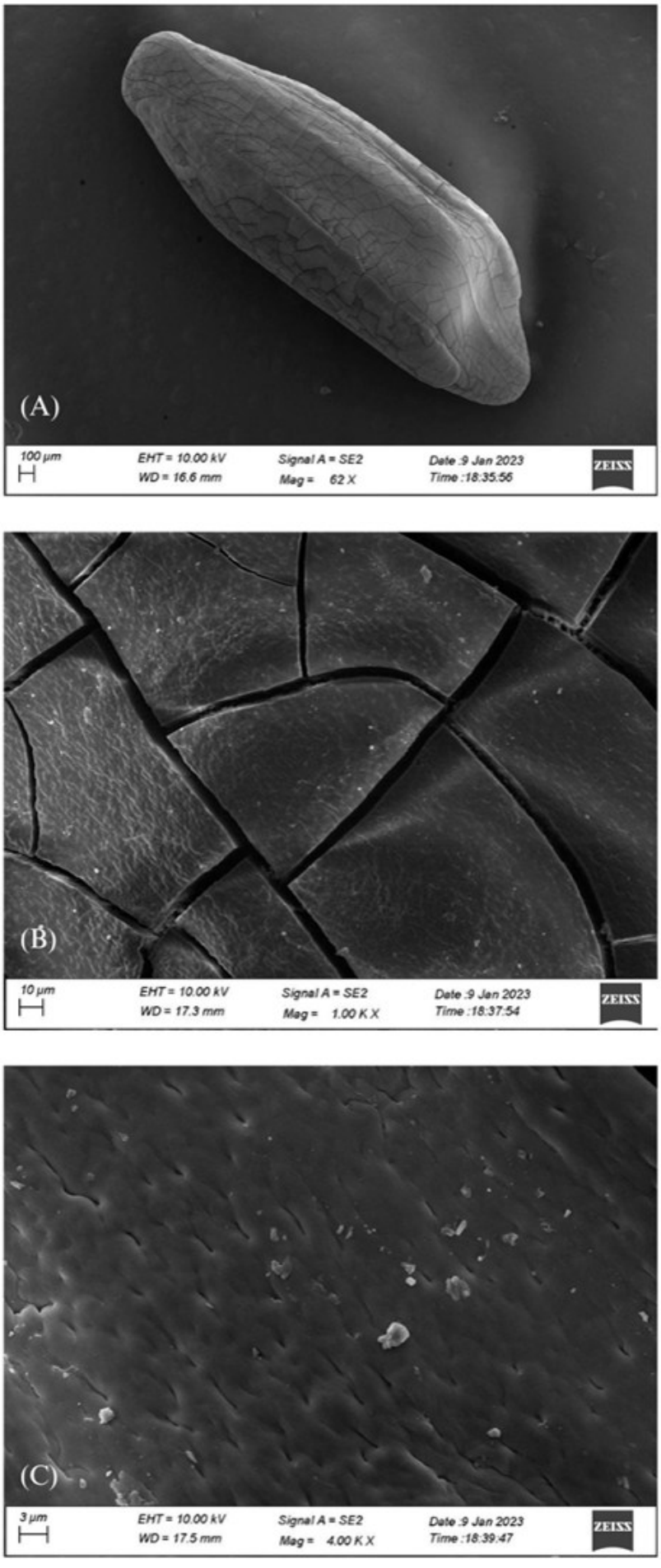


Fig. 10. Scanning electron microscopy (SEM) micrographs of seed morphology and ornamentation of *Senna tora*. A: Magnification: 62X, Scale bar: 100 µm; B: Magnification: 1000X, Scale bar: 10 µm; C: Magnification: 4000X, Scale bar: 3 µm.

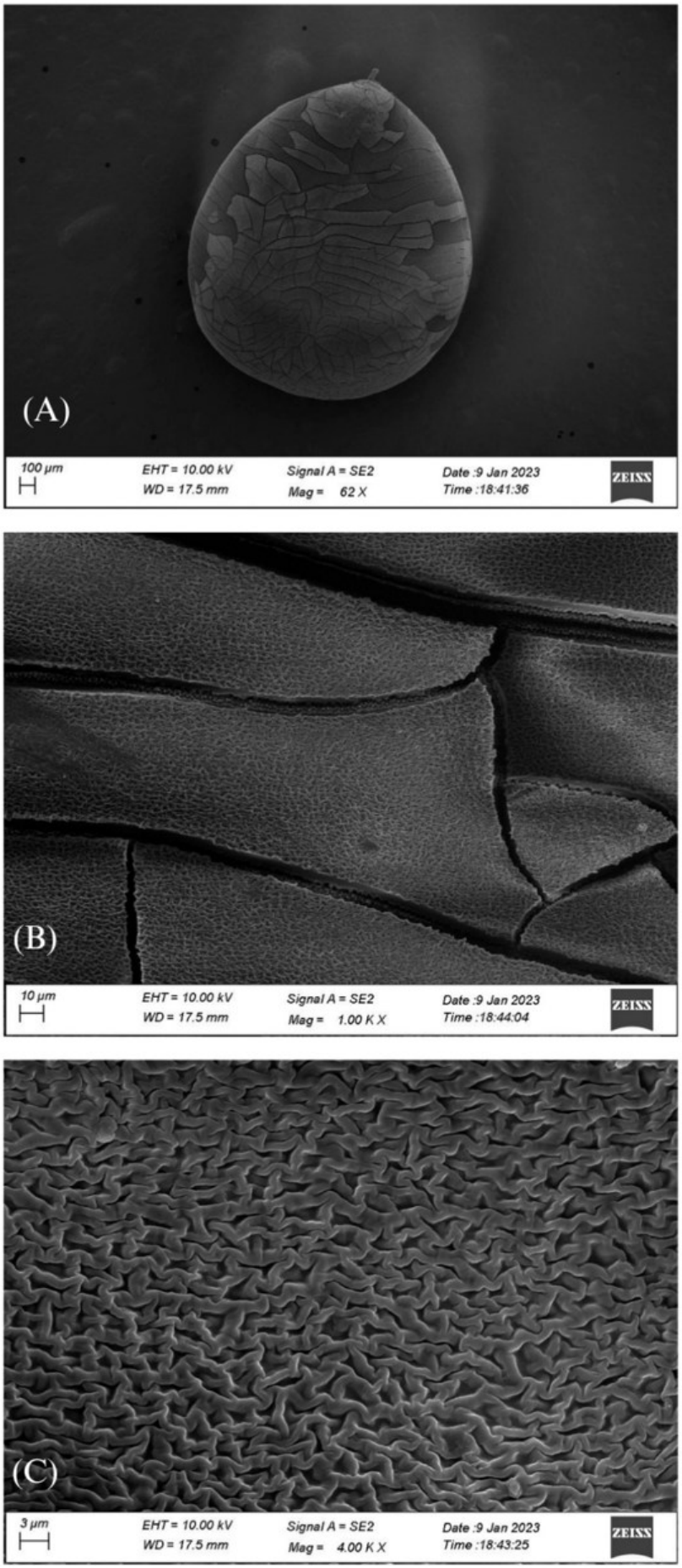


Fig. 11. Scanning electron microscopy (SEM) micrographs of seed morphology and ornamentation of *Senna hirsuta*. A: Magnification: 62X, Scale bar: 100 µm; B: Magnification: 1000X, Scale bar: 10 µm; C: Magnification: 4000X, Scale bar: 3 µm.

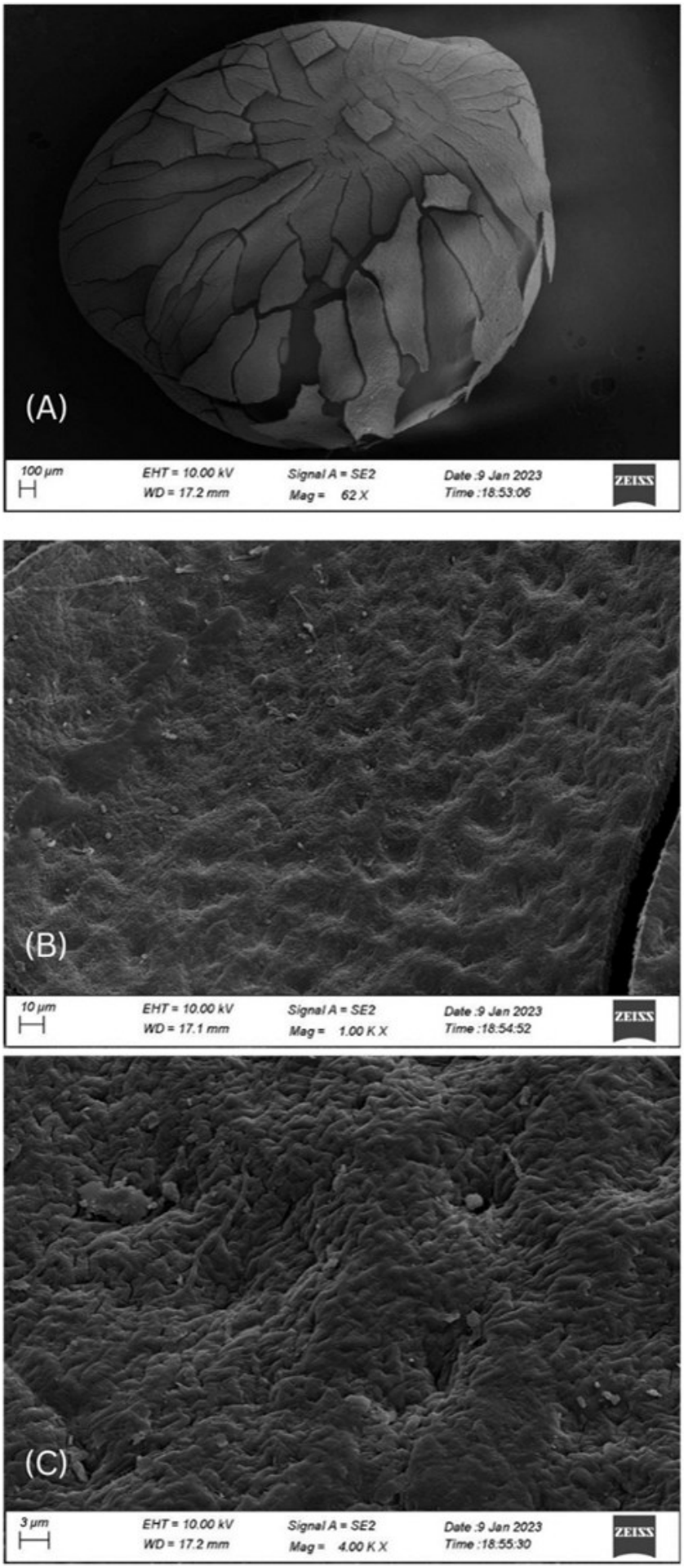


Fig. 12. Scanning electron microscopy (SEM) micrographs of seed morphology and ornamentation of *Senna sophera.* A: Magnification: 62X, Scale bar: 100 µm; B: Magnification: 1000X, Scale bar: 10 µm; C: Magnification: 4000X, Scale bar: 3 µm.

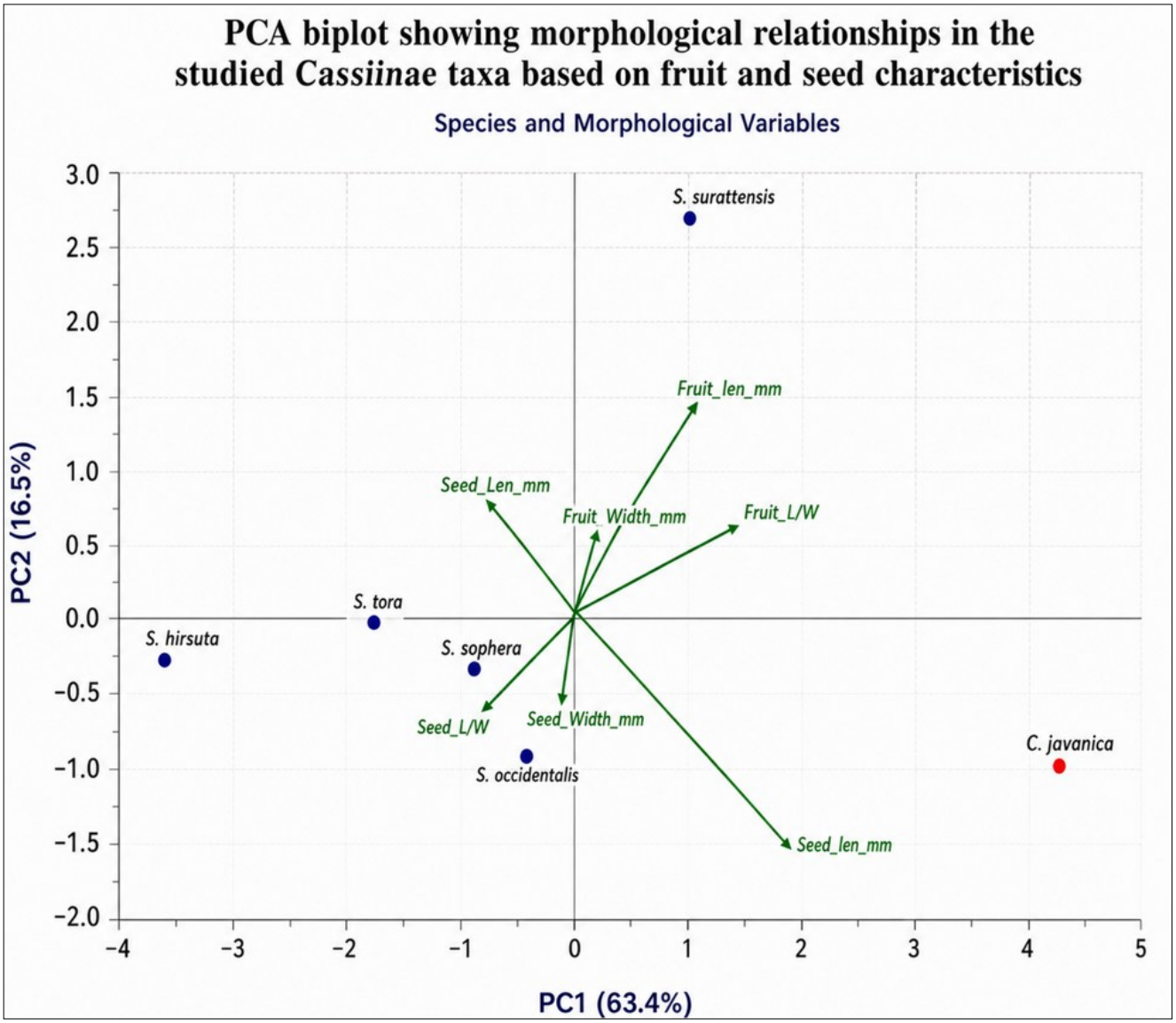


Fig. 13. Principal component analysis (PCA) biplot showing morphological relationships in the studied Cassiinae taxa based on fruit and seed characteristics.

dimensions. At the same time, *S. hirsuta* exhibited the most negative PC1 score (-3.42), consistent with its small seed size. *Senna surattensis* showed distinctive PC2 scores (2.77), consistent with its elongated seed morphology.

Hierarchical cluster analysis using Ward's linkage method produced a dendrogram with high cophenetic correlation (0.768), indicating a reliable cluster structure (Fig. 14). The research revealed *S. occidentalis* and *S. sophera* as the most morphologically similar species (Euclidean distance = 2.26). On the other hand, *C. javanica* and *S. hirsuta* were most dissimilar (distance = 8.22). Three-group clustering separated: (1) a core *Senna* cluster (*S. occidentalis*, *S. tora*, *S. hirsuta*, *S. sophera*), (2) *C. javanica* as morphologically distinct and (3) *S. surattensis* as intermediate.

Dichotomous key to six species of *Senna* and *Cassia* from Assam

1a. Pods indehiscent, large, more than 25 cm long, cylindrical; seeds broadly ovate, heavy; seed surface with thick, fissured cuticular ornamentation under SEM . . . *C. javanica*

1b. Pods dehiscent; seeds lighter, not broadly ovate; seed surface ornamentation variable under SEM . . . 2

2a. Pods subterete, curved, densely strigose; seeds very small, obovate; seed surface distinctly ornamented under SEM; seeds numerous per pod . . . *S. hirsuta*

2b. Pods glabrous or sparsely pubescent; seeds larger than those of *S. hirsuta*; seed surface ornamentation tuberculate, reticulate, or irregular under SEM . . . 3

3a. Seeds highly elongated; pods flat and strap-shaped; seed coat showing diagnostic surface sculpturing under SEM . . . *S. surattensis*

3b. Seeds not highly elongated; pods linear-cylindrical or slightly curved; seed coat ornamentation tuberculate or irregular under SEM . . . 4

4a. Pods flat or slightly curved; seeds medium-sized; seed surface distinctly tuberculate under SEM . . . *S. occidentalis*

4b. Pods strictly linear-cylindrical; seeds shorter; seed surface tuberculate or irregularly ornamented under SEM . . . 5

5a. Seeds rhomboidal, approximately 3 × 2 mm; pods about 12 × 0.6 cm; seed surface tuberculate under SEM . . . *S. tora*

5b. Seeds ovate to suborbicular, approximately 4 × 3 mm; pods about 11 × 0.8 cm; seed surface tuberculate under SEM . . . *S. sophera*

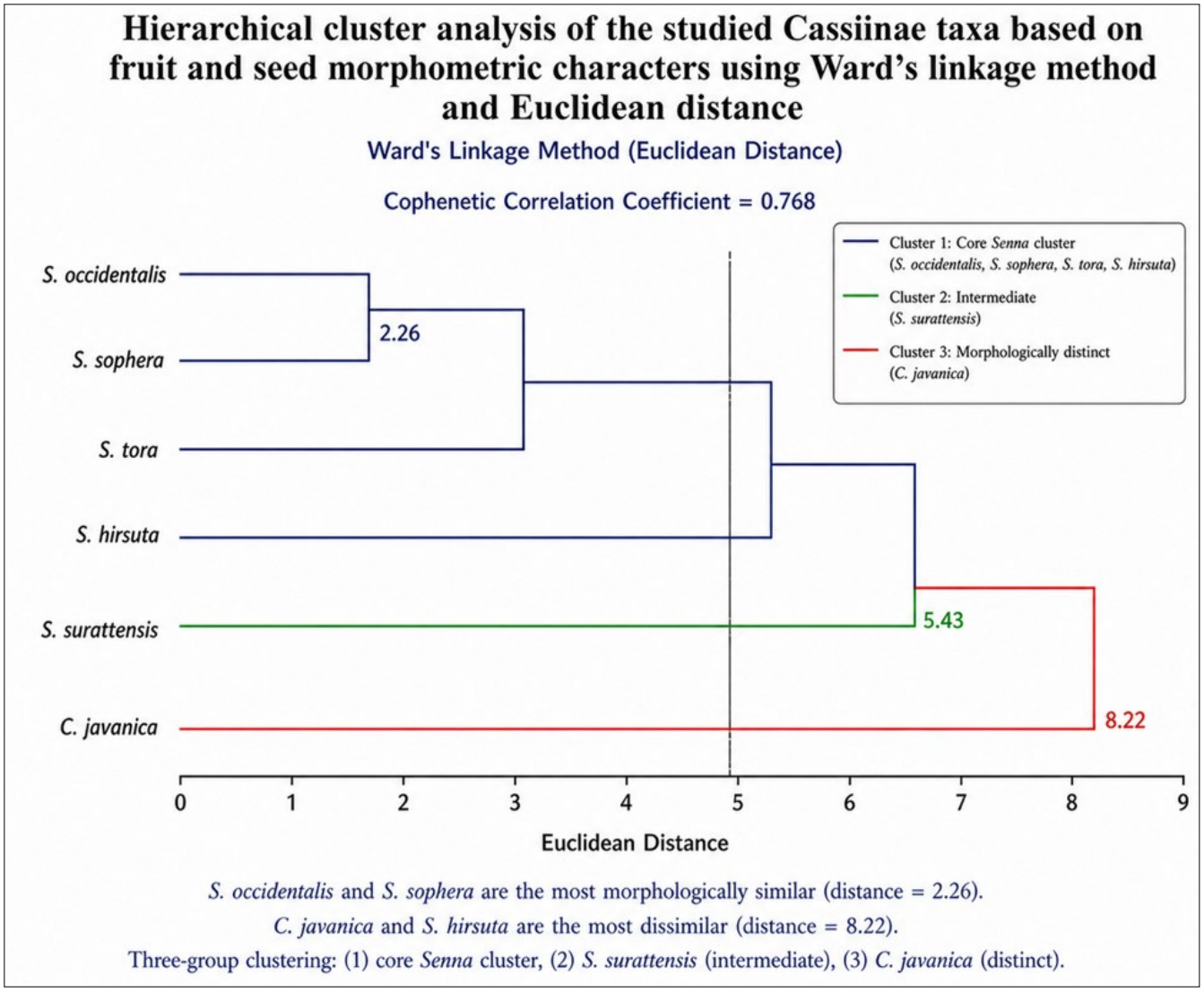


Fig. 14. Hierarchical cluster analysis of the studied Cassiinae taxa based on fruit and seed morphometric characters using Ward's linkage method and Euclidean distance.

Discussion

The results demonstrate that fruit and seed morphology provide reliable diagnostic characters for species delimitation within *Senna* and *Cassia*. In particular, pod dehiscence serves as a key distinguishing character, with *Cassia* typically exhibiting indehiscent pods, whereas *Senna* species possess dehiscent pods. This distinction supports the taxonomic revision that elevated many former *Cassia* species to the genus *Senna* (23). This morphological distinction is supported by molecular phylogenetic studies that validate the monophyly of these genera (24).

Within *Senna*, pod slenderness ratios and seed morphometric parameters provide additional systematic resolution. The highest pod slenderness of *S. hirsuta* (L/W = 29.00) indicates it from morphologically similar species, while seed size and shape characters effectively separate closely related taxa. These findings support previous studies demonstrating the systematic value of fruit and seed characters in Fabaceae (25–27).

Scanning electron microscopy was employed to investigate seed coat micromorphology at high resolution, as many diagnostically important surface features are not discernible under light microscopy. Scanning electron microscopy analysis enabled the detailed characterisation of spermoderm features such as reticulate patterns, tuberculation, cuticular fissures and surface regularity. The use of SEM thus provided high-resolution structural data that complemented macromorphological observations and strengthened the reliability of species discrimination in the present study.

The SEM based research indicated that spermoderm ornamentation patterns show robust concordance with established taxonomic boundaries. The seed-coat micromorphology of the studied taxa showed clear variation, including reticulate ornamentation in *C. javanica* and *S. hirsuta*, tuberculate ornamentation in *S. occidentalis*, *S. tora* and *S. sophera* and a smooth glossy surface in *S. surattensis*. However, whether these characters represent ancestral or derived states cannot be determined without phylogenetic character-state analysis. In contrast, the tuberculate patterns observed in *S. occidentalis*, *S. sophera* and *S. tora,* where the seed surface showed compact and regular tubercles. These interpretations are consistent with comparative developmental and morphological analysis within Cassiinae and related legume taxa (28).

The unique glossy, regular surface of *S. surattensis* seeds suggests possible adaptation to specific dispersal or germination requirements, warranting further ecological investigation. Similar correlations between seed surface morphology and ecological adaptations have been documented in other legume genera (29, 30).

Functional significance of seed coat micromorphology

Seed coat micromorphology in legumes is widely recognised as functionally linked to dormancy regulation, water permeability and protection against environmental stress. The thick, fissured cuticle observed in *C. javanica* may contribute to physical dormancy (hardseededness) by restricting water absorption, a mechanism commonly reported in Fabaceae, where impermeable palisade layers and cuticular modifications delay germination until favourable conditions occur (31, 32). In contrast, the tuberculate spermoderm patterns observed in several *Senna* species may increase surface roughness, potentially fascinating, water observations, soil anchorage or adherence to dispersal agents, as suggested in other legume taxa (33, 34). Such ornamentations can also enhance mechanical protection against abrasion and predation, while microrelief structures may influence interaction with soil microbes and moisture retention.

Additionally, variations in seed surface architecture have been linked to ecological strategies, in which smoother or glossier surfaces (e.g., *S. surattensis*) may promote rapid water uptake and germination, whereas more complex or thickened surfaces are associated with delayed germination and persistence in soil seed banks (33). These functional interpretations suggest that the observed micromorphological diversity in *Senna* and *Cassia* is not only taxonomically informative but also reflects adaptive responses to habitat conditions and regeneration strategies.

Morphological diversity and ecological correlates

The considerable variation in seed size and number per fruit likely reflects different ecological strategies within the study group. *Cassia javanica*, with large, heavy seeds and indehiscent pods, appears adapted to dormancy and seasonal recruitment patterns typical of deciduous forests. In contrast, *S. hirsuta*, with a large number of small seeds, hairy inflorescence and easily dehiscent pods, exhibits characteristics associated with pioneer species in disturbed habitats (35).

The intermediate characteristics of *S. surattensis*, mainly its elongated seeds and moderate pod size, may represent adaptations to semi-arid environments where efficient dispersal and rapid establishment are advantageous. These morphological-ecological correlations support the concept of reproductive syndrome evolution in legumes (36).

Integration with broader systematic studies

The multivariate analysis results align well with molecular phylogenetic studies of Caesalpinioideae. The clear separation of *Cassia* from *Senna* species in morphological space corresponds to their distinct evolutionary origins (20). Within *Senna*, the morphological clustering patterns partly reflect sectional classifications (2). However, some incongruences suggest that fruit and seed evolution may not always track overall phylogenetic relationships.

The high morphological distinctiveness of *S. hirsuta* and *S. surattensis* in our analysis corresponds to their recognition as morphologically and ecologically distinct taxa in regional floras (37). There is a chance that these six selected species may merit particular protection due to their morphological identity and potential ecological roles.

Implications for conservation

The morphological characters identified in this study provide useful diagnostic evidence for species identification, particularly in taxonomic, conservation and restoration contexts. The combination of pod dehiscence, seed size and spermoderm ornamentation patterns provides reliable diagnostic criteria for distinguishing the studied taxa. These characters are particularly valuable when flowering material is unavailable. These characters could be incorporated into regional identification keys used in floristic surveys, seed collection and forest restoration programs in Assam and surrounding regions.

These morphological points examined here also inform its protection as a part of the Indo-Burma biodiversity hotspot for legume diversity. By improving species-level identification, these morphological and SEM-based characters strengthen taxonomic documentation and provide diagnostic evidence for future conservation assessments of the studied *Senna* and *Cassia* taxa within the Indo-Burma biodiversity hotspot.

Limitations and future directions

This study provides useful fruit, seed and SEM-based spermoderm characters; however, several limitations should be acknowledged. The limited taxonomic sampling and restricted geographic coverage of the present study, which included six species from Assam, constrain broader generalisations about fruit, seed and spermoderm variations in *Senna* and *Cassia*. Future studies should include wider taxonomic and geographic sampling, including additional regional species and populations from different ecological zones.

Integration of these morphological data with molecular phylogenetic evidence would help clarify the systematic and evolutionary significance of the observed characters. Character mapping on molecular phylogenies could help determine whether similar morphological traits reflect shared ancestry or convergent evolution within these genera.

Conclusion

This study demonstrates that fruit and seed morphology, particularly pod dehiscence, size ratios, seed dimensions, spermoderm patterns (reticulate vs tuberculate) and 100-seed mass, provide reliable diagnostic features for distinguishing *Cassia javanica* from *Senna* species and delimiting species within *Senna*. Scanning electron microscopy (SEM) and multivariate analysis successfully discriminated all six taxa, explaining 92.1 % of the variation. The findings support the taxonomic utility of these characters in Caesalpinioideae systematics and offer practical identification tools. However, the limited sampling restricts broad evolutionary interpretations and integration with molecular data and broader taxon sampling is recommended for deeper insights.

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Authors' contributions

RT conceptualised the study and designed the research framework. MB carried out field sampling and specimen collection, performed macromorphological measurements, conducted scanning electron microscopy (SEM) based micromorphological observations, analysed the data and performed the statistical analysis. RT and MB contributed to the drafting of the manuscript. RT and BND critically revised the manuscript and approved the final version. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

Ethical issues: None

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work, the authors used ChatGPT (OpenAI) for language editing, grammar correction, formatting assistance, figure-caption polishing and preparation of response/report documents. After using this tool/service, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

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