Seed Coat Micromorphology of Sida Species from the Deccan Plateau and its Taxonomic Implications[1]

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ABSTRACT

Sida L. (Malvaceae: Malvoideae: Malveae) is a large, mainly tropical genus of herbs and subshrubs. In India, several of its species are common weeds and several others are used in traditional medicine. The species are often difficult to separate on vegetative characters, and new species continue to be described from the country. The surface sculpture of the seed coat (testa), resolved in detail by scanning electron microscopy (SEM), has proved taxonomically informative in many angiosperm groups, and earlier surveys of Malvaceae that included a few *Sida* species suggest that testa patterns differ among species. However, no systematic SEM study of *Sida* seeds from the Deccan Plateau, based on several populations per species, has been published. This protocol describes a study that will document seed size, shape, colour, hilum characters and testa sculpture in the *Sida* species of the Deccan Plateau in Telangana and adjacent regions, quantify variation within and among species, and evaluate the diagnostic value of these characters. Mature seeds will be collected from vouchered populations, described under a stereomicroscope and imaged by SEM at three fixed positions. Characters will be scored with standard terminology and analysed by multivariate methods, and the stable characters will be used to construct a provisional identification key to seeds.

Keywords: *Sida; Malvaceae; seed coat; testa sculpture; scanning electron microscopy; spermoderm; taxonomy; Deccan Plateau*

1. Introduction

Seed surface characters have been used widely in angiosperm systematics since scanning electron microscopy made the fine sculpture of the seed coat readily accessible. In an influential review, Barthlott (1981) showed that epidermal and seed surface characters frequently carry systematic information at several taxonomic levels, the most informative being the shape of the testa cells, the relief of their outer walls and the fine sculpture of the cuticle. Seed micromorphology has since been applied to many families, including Malvaceae.

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1.1 Seed surface studies in the mallow family

Molecular evidence from plastid DNA supports a broadly circumscribed Malvaceae that includes the former Bombacaceae, Sterculiaceae and Tiliaceae (Bayer et al. 1999). Within subfamily Malvoideae, several studies have examined seed characters; those most relevant to the present work are summarised in Table 1. An SEM survey of seed surfaces in Indian Malvaceae, which included species of *Sida*, recorded a range of testa patterns (Paul & Nayar 1987). The systematic implications of seed-coat morphology were discussed for Egyptian Malvaceae (El Naggar 2001), and in the *Sida ovata* complex in Pakistan, seed micromorphology and seed proteins were used as evidence of hybridisation (Abid et al. 2014).

In India, the seed and seedling morphology of ten medicinal Malvaceae, including *Sida*, was described from Tripura, where the characters were used to construct an artificial key (Rani & Datta 2020).

Table 1. Published seed studies of *Sida* and related Malvaceae used as background, summarised from the cited articles.

Study	Taxa and region	Methods	Main findings
Paul & Nayar 1987	Some taxa of Indian Malvaceae, including *Sida*	SEM of seed surface	Survey of testa surface patterns in Indian Malvaceae, including *Sida*
El Naggar 2001	Malvaceae, Egypt	Seed-coat form	Systematic implications of seed-coat characters discussed
Abid et al. 2014	*Sida ovata* complex, Pakistan	Seed surface detail and seed proteins	Seed micromorphology used as evidence of hybridisation
Rani & Datta 2020	10 medicinal Malvaceae species, Tripura	Seed and seedling form	Artificial key based on seed and seedling characters
Tambde et al. 2020	*Sida sivarajanii* (new species), Aurangabad, Maharashtra	Plant form, SEM of mericarps and seeds, ITS DNA	Multireticulate spermoderm pattern distinguished the new species from *S. alnifolia* and *S. rhomboidea*

1.2 *Sida* in India, and why seed features would help

Sida is a taxonomically difficult genus. Species are distinguished mainly by leaf shape, indumentum, stipules, inflorescence structure and the mericarps (the segments into which the fruit separates), and many of these characters vary with habitat. New species continue to be described from peninsular India (Sivarajan & Pradeep 1990; Tambde et al. 2020). *Sida sivarajanii*, described from Aurangabad, Maharashtra, was distinguished from its closest relatives, *S. alnifolia* and *S. rhomboidea*, partly by its multireticulate spermoderm pattern, and its distinctness was supported by ITS sequence data (Tambde et al. 2020). This example indicates the potential of seed characters in the genus. The seed coat is also biologically important; in *S. spinosa*, it forms a water-impermeable barrier that governs dormancy (Egley & Paul 1993).

Several *Sida* species are used in Indian traditional medicine, and the crude drug traded as 'bala' (*S. cordifolia*) may include material of other *Sida* species. Mericarp morphology has been proposed as a practical aid to the identification of Indian *Sida* (Bharati 2016). Reliable characters for identifying seeds and fruits are therefore useful beyond taxonomy. Seed characters would complement those of the mericarp, because seeds remain available after mericarps break up and the testa can be examined on very small samples.

1.3 Knowledge gap and novelty of the study

Existing Indian studies of *Sida* seeds either survey Malvaceae broadly and include only a few *Sida* species (Paul & Nayar 1987), describe seeds largely by light microscopy within a regional flora (Rani & Datta 2020) or concern a single new species (Tambde et al. 2020). Studies from other countries (El Naggar 2001; Abid et al. 2014) deal partly with different species. To our knowledge, testa micromorphology has not been compared across the *Sida* species of the Deccan Plateau using several populations per species.

The present study differs from earlier work in four respects: it concentrates on the *Sida* flora of the Deccan Plateau; it samples several populations of each species so that intraspecific variation can be quantified; it images fixed positions on each seed following a standard protocol; and it tests explicitly whether seed characters separate species recognised on other evidence.

1.4 Aims, questions and hypotheses

The study aims to describe the gross morphology and testa micromorphology of Deccan *Sida* seeds, to quantify variation within and among species, and to assess the taxonomic value of seed characters through multivariate analysis and a provisional key.

- **Q1.** Which seed characters are constant within species across populations? The characters considered include shape, size, colour, the hilum and its surroundings, indumentum, testa cell pattern, anticlinal wall relief and secondary sculpture.
- **Q2.** Do these characters separate the *Sida* species of the study area, including pairs that are difficult to distinguish vegetatively?
- **Q3.** Are the testa patterns consistent with earlier descriptions of the same species from India and elsewhere (Paul & Nayar 1987; El Naggar 2001; Abid et al. 2014)?

Hypothesis H1: the primary testa pattern (the shape of the testa cells and the relief of the anticlinal walls between them) will be constant within species and will differ among at least some species, whereas seed size will overlap more widely among species.

2. Materials and Methods

2.1 Study region

The Deccan Plateau occupies most of peninsular India south of the Vindhya and Satpura ranges. The core study area is Telangana, particularly the semi-arid southern districts, including Nalgonda; adjacent parts of the plateau may be included if additional species are found there. The climate is semi-arid and tropical, with monsoon rainfall and a prolonged dry season. *Sida* is expected mainly on disturbed ground, roadsides, field margins, fallow land, scrub and forest edges. Collection localities will be reported after fieldwork.

2.2 Species and sampling plan

The list of target species will be compiled from regional floras, recent Indian taxonomic work on *Sida* (Tambde et al. 2020) and herbarium records, and will then be confirmed in the field. Species widely reported from peninsular India, such as *S. acuta* Burm.f., *S. cordifolia* L., *S. cordata* (Burm.f.) Borss.Waalk., *S. rhombifolia* L. and *S. spinosa* L., will be included when they are located and their identity is confirmed. The final species list will depend on the material collected and verified.

For each species, three to five populations, at least 10 km apart where possible, will be sampled, with seeds collected from at least five plants per population (Table 2). Only mature seeds from dehiscing or fully dry mericarps will be used. A herbarium voucher bearing flowers and fruits will be prepared for every sampled plant and deposited in a

recognised herbarium. Identifications will follow current literature and will be checked against authenticated specimens. Where a species cannot be found in the field, seeds from herbarium sheets may be added with the permission of the herbarium concerned.

Table 2. Planned sampling and imaging targets.

Item	Target
Populations per species	3–5, at least 10 km apart where possible
Plants per population	At least 5, each with a voucher
Seeds measured under the stereomicroscope	10 per plant (about 50 per population)
Seeds imaged by SEM	3–5 per plant from at least 2 plants per population (about 10–15 per population)
SEM positions per seed	3 (lateral face, dorsal region, hilar region; Figure 1)
Testa cells measured	About 20 per image
Repeat scoring	A random subset re-scored by a second observer blind to species identity

2.3 Permissions

Seed collection will follow applicable regulations. The work comply with the Biological Diversity Act, 2002. Removal of seeds from herbarium sheets will require the curator's permission. Although many *Sida* species are common weeds, only the material required for the study are collected.

2.4 Seed form under the stereomicroscope

Seeds will be removed from the mericarps, cleaned of debris and air-dried. Ten seeds per plant will be photographed under a stereomicroscope with a scale bar. Length, width and thickness will be measured on calibrated images in ImageJ (Schneider et al. 2012), and seeds will be weighed in batches. Seed outline (for example reniform, trigonous-reniform or ovoid), colour, lustre, the presence and distribution of hairs, the position and shape of the hilum, and the presence of a raised rim or an aril-like outgrowth near the hilum will be recorded.

2.5 Scanning electron microscopy

For SEM, three to five seeds per plant from at least two plants per population will be prepared. Seeds will be cleaned gently with a fine brush and, where necessary, given a brief ultrasonic treatment in water or ethanol after a pilot test has confirmed that the surface is not damaged. They will be air-dried or dried in a desiccator, mounted on aluminium stubs with carbon tape so that both the lateral face and the hilar region are visible, and sputter-coated with gold or gold–palladium.

Images will be taken at standard magnifications: the whole seed, ×200–500 for the testa cell pattern and ×1000–3000 for secondary sculpture. Three fixed positions will be imaged: the centre of the lateral face, the dorsal region and the hilar region (Figure 1). Fixed positions are essential, because testa sculpture can vary over the surface of a seed. The SEM facility will be named once access has been confirmed.

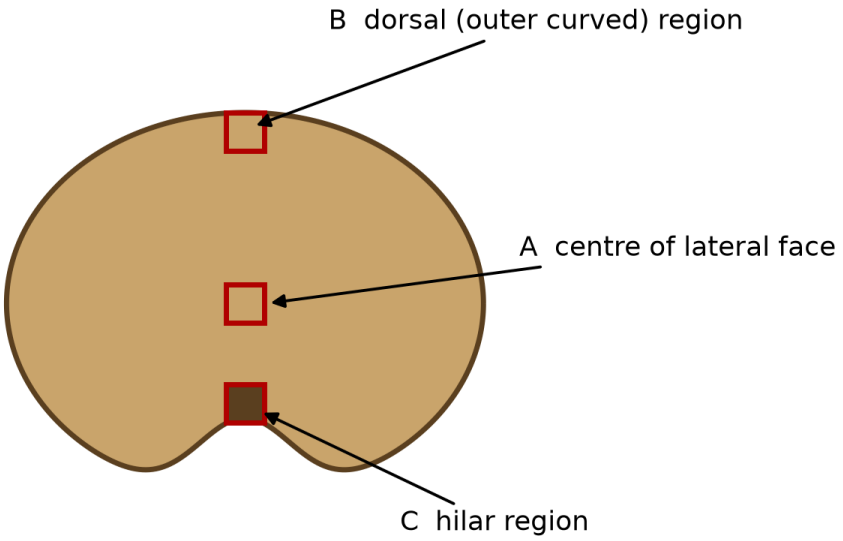


Figure 1. The three fixed SEM imaging positions planned for each seed: A, centre of the lateral face; B, dorsal (outer curved) region; C, hilar region. Schematic diagram of a generalised reniform seed, not to scale.

2.6 Characters and terminology

Testa characters will be scored within the general framework of Barthlott (1981), as summarised in Table 3. Categories of overall pattern will be defined at the outset with reference drawings (Figure 2) so that each category is applied consistently. Seed hairs, if present, will be described with standard trichome terminology (Payne 1978). A random subset of images will be scored independently by a second observer blind to species identity in order to assess the repeatability of scoring.

Table 3. Seed and testa characters to be recorded.

Feature	Definition	Recording method
Seed outline	Outline in lateral view	Reniform, trigonous-reniform, ovoid, etc.
Size and mass	Seed dimensions and mass	Length, width and thickness (µm); batch mass
Hilum	Scar marking the point of attachment of the funicle	Position, shape, any raised rim
Primary sculpture	Shape and arrangement of testa cells	Polygonal, elongated, irregular
Anticlinal walls	Walls between adjacent testa cells	Raised or sunken; straight, curved or wavy; width
Periclinal walls	Outer wall of each testa cell	Flat, dished (concave) or domed (convex)
Secondary sculpture	Fine sculpture superimposed on the cells	Striations, granules, cuticle folds, wax
Overall pattern	General appearance of the surface	Reticulate, rugose, striate, foveate, verrucate (Figure 2)

Testa cell size	Dimensions of individual cells	Length and width of about 20 cells per image

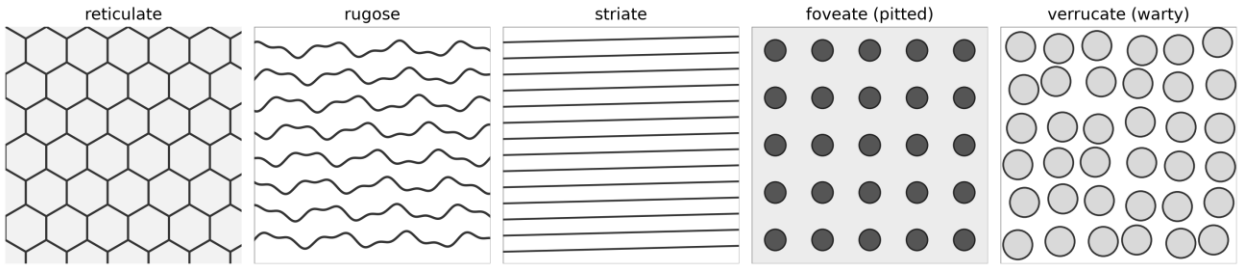


Figure 2. Simplified line drawings of the five overall testa patterns to be used in scoring: reticulate, rugose, striate, foveate (pitted) and verrucate (warty). Line drawings of terms, not SEM images.

2.7 Data analysis

- Seed size will be analysed with linear mixed-effects models, with species as a fixed effect and population as a random effect (Bates et al. 2015), in order to partition variation into within-species and between-species components.
- Qualitative and quantitative characters will be combined in a mixed-data matrix and analysed by principal coordinates analysis of Gower distances and by UPGMA clustering; principal component analysis will be applied to the quantitative characters alone. Cross-validated linear discriminant analysis will be used to test how often seeds are assigned to the correct species on the basis of seed characters alone.
- Characters that are constant within species but differ among species will be used to construct a provisional dichotomous key to seeds, which will then be tested on seeds from specimens not used in its construction.
- The results will be compared with published testa descriptions for the same species (Paul & Nayar 1987; El Naggar 2001; Abid et al. 2014; Rani & Datta 2020).

2.8 Mericarp characters

Many of the characters used in *Sida* keys are borne on the mericarps: their number, the presence and length of apical awns, the reticulation of the dorsal and lateral walls, and the mode of dehiscence. Mericarp morphology has also been proposed for identifying Indian *Sida* (Bharati 2016). Mericarps from the same plants as the seeds will therefore be photographed and scored, so that it can be determined whether seed characters add information to that provided by the mericarps or largely duplicate it. The two data sets will be analysed separately and in combination, and the gain in correct identification from adding seed characters will be reported.

2.9 Sample size

The targets of three to five populations and five plants per population per species balance the effort required for SEM against the need to capture intraspecific variation. Because seeds from one plant are not independent, the plant will be the unit of replication in statistical analyses, with seed values averaged or treated as nested. Species represented by fewer than three populations will be described without formal testing, and the results will be identified as provisional.

2.10 Anticipated limitations

Some species may be found in only a few populations. Because maturity strongly affects seed colour and surface appearance, only fully mature seeds will be used and their maturity will be recorded. Cleaning may damage delicate surface structures, so cleaning methods will be compared in a pilot study. Hybridisation, which has been reported in the *Sida ovata* complex (Abid et al. 2014), could produce intermediate patterns.

3. Expected outcomes

If H1 is supported, the primary testa pattern and anticlinal wall relief will prove stable within species and will separate at least some species, while seed size will be of limited value because of overlap. Such characters would form the basis of a seed key that can be applied to fragmentary material, including dispersed seeds and herbal raw material. If testa patterns vary substantially among populations, the study will instead document the extent of this variation and identify which characters should be used cautiously in *Sida* taxonomy.

4. Anticipated discussion points

- Which seed and testa characters remain constant within species, and which vary among populations.
- Whether seed characters separate the *Sida* species of the Deccan Plateau, including morphologically similar pairs.
- How the testa patterns compare with earlier descriptions from India and other regions (Paul & Nayar 1987; El Naggar 2001; Abid et al. 2014; Rani & Datta 2020) and with the spermoderm pattern reported for *S. sivarajanii* (Tambde et al. 2020).
- Whether the provisional key can assist in identifying seeds or fruits in herbal raw material and in seed-bank studies (Bharati 2016).
- Whether testa structure is linked to seed dormancy and water impermeability (Egley & Paul 1993).
- Limitations of the study and future work, including the integration of seed data with molecular evidence.

Declarations

Funding: No specific funding has been received for this study.

Competing interests: The author declares no competing interests.

Author contributions: The author. conceived and designed the study and wrote the protocol.

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