Seed coat architecture of four Indian species of *Ephedra* and its taxonomic significance

Structural details of seed coat have proved universally useful in solving systematic problems, establishing evolutionary relationships, segregating hybrids and in the identification of scattered fragments in palaeobotanical studies[1–3].

Knowledge of seed coat morphology and ultrastructure of angiosperms though widely known, there is lack of information on the gymnosperms. In the classification of gymnosperms, in particular, the use of epidermal characters has received wide recognition. The importance of epidermal characters in delimitating Pteridospermales, Bennettitales, Cycadales, Coniferales, Taxales and Ginkgoales, and the significance of epidermal and cuticular features of the stem and leaf of 24 species of the genus *Ephedra* and its taxonomic importance have been emphasized[4]. Seed morphology and anatomy of *Phyllocladus*[5] and *Austrotaxus spicata*[6] were studied for the analysis of their systematic position and phylogenetic relations. The importance of seed coat structure and its taxonomic significance in some species of *Cupressus* is evident[7]. However, structural organization of *Ephedra* seed coat has not been studied so far. Considering this lacunae, the present study was carried out to elucidate in detail, micromorphological and ultrastructural characteristics of seed coat of four Indian species of *Ephedra* for correct identification and construct an artificial key.

Comprising about 50–60 species, *Ephedra* is the only genus belonging to the family Ephedraceae and order Ephedrales[8]. It is widely distributed in the arid regions of the New and Old World, from the Mediterranean and Black Sea shores up to China, including northern and North East India. The plants are mostly shrubs, show extreme xerophytic characters and have enormous therapeutic value[9].

Mature seeds from dry cones of *Ephedra foliata*, *E. intermedia*, *E. saxatilis* and *E. gerardiana* were collected from three phytogeographical regions of India (Table 1). Fifty healthy seeds of each species were randomly selected and examined. Morphological study, observation and photography were done under incident light microscope (Zeiss Stemi SV 11, Geological Survey of India,

Table 1. Sampling site of the studied Indian species of *Ephedra*

Species studied	Locality	Latitude/longitude	Altitude (m)	Rainfall (mm)	Temperature (°C)	Soil condition
Ephedra intermedia	Himachal Pradesh	29°28′N–79°39′E	2213	1500–1750	0–15 in winter, 22–33 in summer	Grey brown podzolic
Ephedra gerardiana, *Ephedra saxatilis*	Sikkim	27°03′N–88°57′E	6000	2000–5000	3–12 in winter, 22–30 in summer	Dark brown loamy soil
Ephedra foliata	Haryana	30°11′N–69°00′E	1219	100–165	8–18 in winter, 32–48 in summer	Sandy desert soil

Table 2. Comparative morphological features of seed coat of *E. saxatilis*, *E. gerardiana*, *E. intermedia* and *E. foliata* (see Figure 1 *a–p*)

Characters	*E. saxatilis*	*E. gerardiana*	*E. intermedia*	*E. foliata*
Seed coat texture	Surface ridged, usually exerted	Surface slightly ridged	Surface almost smooth, slightly striated	Surface smooth, longitudinally striated
Seed coat architecture	Epidermal cells densely reticulate, ridges and furrows well developed, distantly placed, ridges exerted, apex uniformly ornamented as the seed coat surface	Epidermal cells densely reticulate, ridges and furrows well developed, closely placed and grooved, apex uniformly ornamented as the seed coat surface	Reticulum of epidermal cells poor, almost smooth, longitudinally striated, ridges and furrows closely placed, shallow, appearing wavy in nature, cells of the apical region shorter and compactly arranged than the remainder of the seed coat	Epidermal cells deeply ridged, furrows uneven, distantly placed, appearing like coiled ropes, apex composed of loosely arranged cells
Epidermal cell structure	Cells elongated, rectangular, walls raised	Cells rhomboidal to polyhedral, walls depressed	Cells elongated, rectangular with oblique transverse walls, some with tapering ends, walls smooth	Cells elongated, rectangular, fibrillar, coiled thread-like
Anticlinal wall shape	Grooved	Grooved	Flat	Grooved
Anticlinal wall texture	Convex	Concave	Not conspicuous	Not conspicuous
Periclinal wall texture	Wavy	Serrated or dentate	Almost smooth	Undulated
Epicuticular wax deposition	Present as minute platelets	Present as minute platelets	Present uniformly, amorphous in nature	Present as minute platelets

Table 3. Comparative anatomical features of seed coat of *E. saxatilis*, *E. gerardiana*, *E. intermedia* and *E. foliata* (see Figure 2 *a–h*)

Characters	*E. saxatilis*	*E. gerardiana*	*E. intermedia*	*E. foliata*
Mesotesta	Parenchymatous, containing profuse tracheary elements with spiral thickening	Parenchymatous, containing profuse tracheary elements with spiral thickening	Parenchymatous, containing profuse starch grains, vasculature absent	Parenchymatous, containing profuse starch grains, vasculature absent

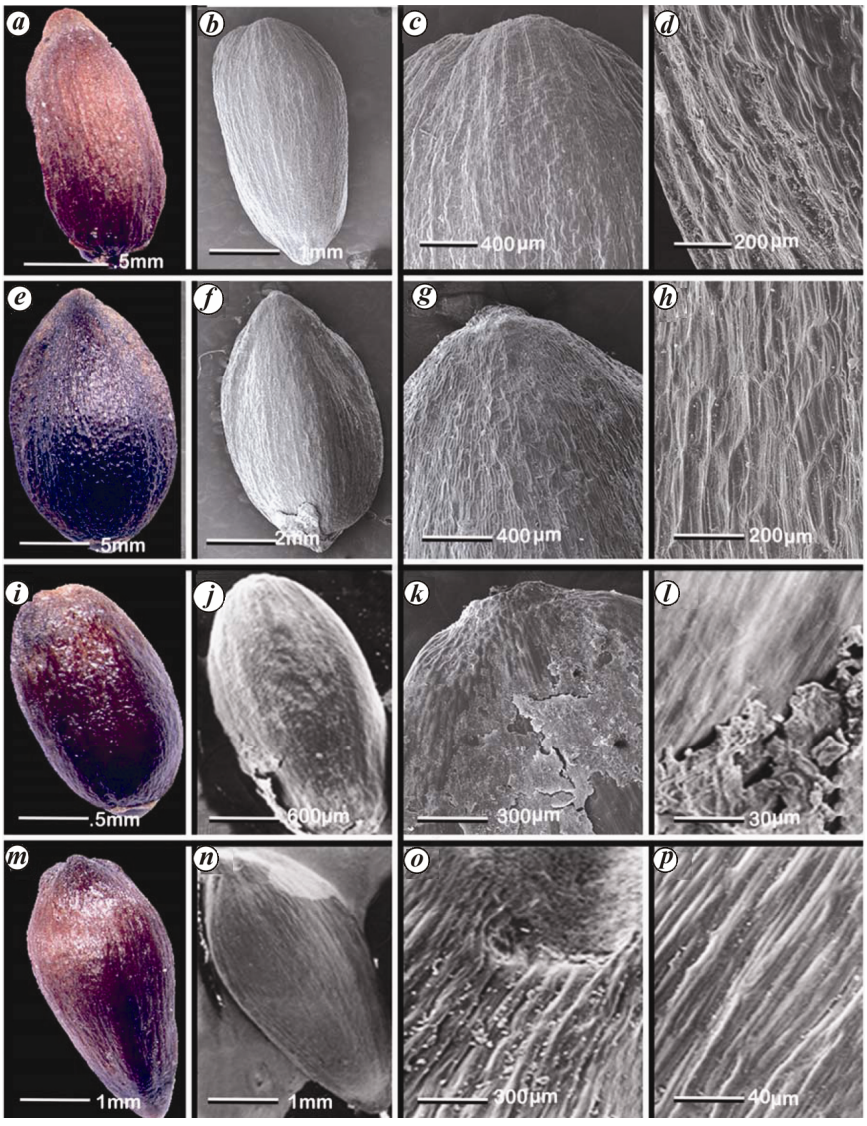

Figure 1. Morphological features of seed coat of *Ephedra saxatilis*, *E. gerardiana*, *E. intermedia* and *E. foliata* under light microscope (LM) and scanning electron microscope (SEM). ***a***, External morphology of *E. saxatilis* seed under LM; ***b***, Same under SEM showing prominent median depression at the apex and surface with ridges and furrows; ***c***, Magnified view showing distantly placed ridges and furrows; ***d***, Surface enlarged showing cell pattern; ***e***, External morphology of *E. gerardiana* under LM; ***f***, Same under SEM showing nature of apical portion and surface with ridges and furrows; ***g***, Ridges and furrows of seed surface and apical portion magnified; ***h***, Rhomboidal to polyhedral cells with depressed walls; ***i***, External morphology of *E. intermedia* under LM; ***j***, Same under SEM showing closely placed cells; ***k***, Ornamentation of apical portion of seed coat showing shorter and compactly arranged cells; ***l***, Seed coat showing elongated, rectangular cells with oblique transverse walls, tapering ends and amorphous wax on its surface; ***m***, External morphology of *E. foliata* under LM; ***n***, Same under SEM showing apical depression; ***o***, Surface with deeply lobed ridges and furrows; ***p***, Elongated rectangular and fibrillar cells appearing as coiled thread like structure.

Kolkata). For scanning electron microscope (SEM) study, seeds were air-dried, mounted on aluminium stubs with glue, coated with palladium in a sputter coater and scanned under SEM. Photographs were taken using Leica S440 SEM at an accelerating voltage of 15 kV. For anatomical study, thin transverse sections (TS) and longitudinal sections (LS) were made, observed, studied and photographed under transmitted light microscope (Zeiss Axioskop 2, University of Calcutta, Kolkata).

Comparative analysis of the seed morphology and ultrastructural features of the seed coat reveals that they can be morphologically distinguished on the basis of the number of seeds per cone, seed size, shape, weight, colour, seed coat texture, seed surface architecture, epidermal cell shape, pattern, size, anticlinal wall shape, anticlinal wall level, anticlinal wall thickness, periclinal wall level and periclinal wall texture, vasculature type and cell depositions (Tables 2 and 3; Figures 1 and 2). Ultrastructure reveals well-defined differences in the presence or absence of ridges and furrows, closely or distantly placed, shape, size, pattern and alignment of the epidermal cells, presence or absence of epicuticular wax (Figure 1 *a–p*), vasculature and starch grains (Figure 2 *a–h*).

Salient features of the seed coat of Ephedraceae observed in the present study are: presence of epicuticular wax, well-developed cuticle, reticulations, ridges and furrows, occurrence of vasculature and starch grains.

Seeds are deep chocolate, obovoid to pyriform, basal end tapering, pointed, apex slightly depressed, pit-like, surface with raised verrucose lines in *E. foliata*, deep chocolate, ellipsoidal to oblong, pyriform and ovoid, apex slightly elevated, notched at the centre in *E. intermedia*, greyish-brown, ellipsoidal to ovoid, apex obtuse or subacuminate in *E. gerardiana*, while in *E. saxatilis* they are greyish-black oblong to pyriform, apex slightly bilobed, with median

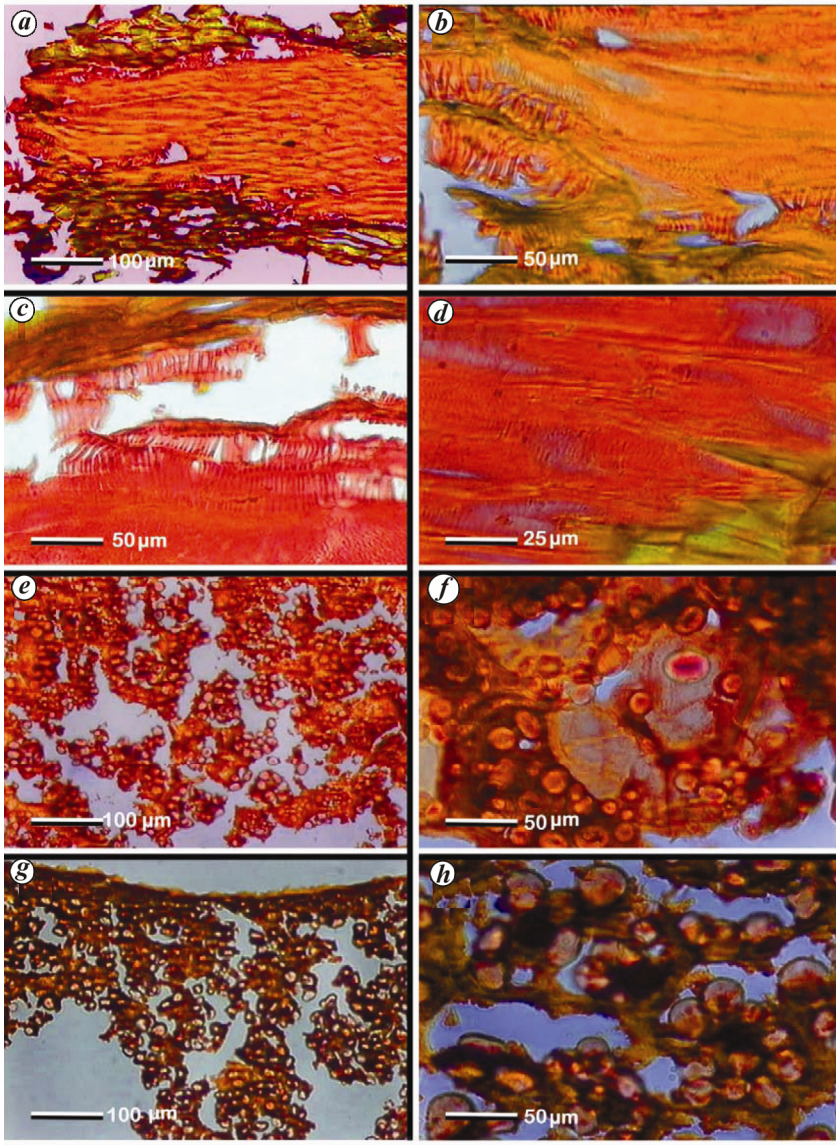

Figure 2. Anatomical features of seed coat of *E. saxatilis*, *E. gerardiana*, *E. intermedia* and *E. foliata* under LM. ***a***, Longitudinal section (LS) of seed coat of *E. saxatilis* showing cuticle (arrow) and seed coat layers; ***b***, Same magnified showing vasculature and tracheidal thickenings (arrow); ***c***, LS of seed coat of *E. gerardiana*, showing cuticle (arrow), seed coat layers and vasculature; ***d***, A portion enlarged showing cell pattern and tracheidal thickenings (arrow); ***e***, LS of seed coat of *E. intermedia* showing mesotesta containing profuse starch grains; ***f***, Starch grains magnified (arrow) showing solitary nature; ***g***, LS of seed coat of *E. foliata* showing cuticle, seed coat layers and starch grains; ***h***, Aggregated starch grains magnified (arrow).

furrow. Seed coat surface of all the taxa studied are cutinized (Figure 1 *a–p*).

Anatomically in all the four species the seed coat is differentiated into three layers; exotesta, mesotesta and endotesta, with the major portion being occupied by mesotesta. Exotesta is mainly parenchymatous, covered by a thin cuticle in all the studied species, except *E. foliata* where it is thick and made up of 3–4 tiers of cells. Endotesta in all the species is composed of compressed parenchymatous cells (Figure 2 *a–f*).

Morphology of the seed and microsculpturing of the seed coat of four Indian species of *Ephedra* have been investigated under light microscope (LM) and SEM that allows separation of species apparently looking similar under LM. Although seed characters are genetically constant, ecological factors may have an operative role in developing variable ultrastructural features. The data recorded from morphological and anatomical studies and SEM observations (Tables 2 and 3) reveal that the four species differ strikingly in seed coat architecture, epidermal cell character, epicuticular wax deposition, vasculature and cell deposition which have been used to construct an artificial key for specific identification.

The cuticular pattern, orientation of epidermal cells and their shapes are distinct and distinguishable from each other in all the seed coats studied. This particular pattern which is species-specific may have taxonomic significance as well as ecological implication.

All the species studied show different forms of wax deposition on their seed coat. Superhydrophobicity is related to epicuticular wax deposition, which is biologically significant as a mechanism to protect against plant pathogens, because germination and penetration of many microorganisms, such as fungi within the plant tissues and reproduction of bacteria are limited by water access[10]. So the occurrence of wax may be related with the protection of seeds. The sculpturing of a chemically hydrophobic surface with the presence of cutin and wax increases the water repellency[11–15]. Thus, the presence of wax on the seed surface may have the same ecological role.

Anatomically, *E. saxatilis* and *E. gerardiana* show well-developed vasculature, tracheids with various thickenings but no starch grains, whereas *E. intermedia* and *E. foliata* contain profuse starch grains and no vasculature. As the seed contains an embryo, which remains enveloped by the seed coat, the tracheids may help in conduction and provide mechanical support to the growing embryo. Abundant starch grains present as readily available stored food for the growing embryo is also an advantage for the germinating seeds.

The present study reveals distinct morphological and anatomical characteristics, which have helped in the separation of species apparently looking similar under LM. It is therefore of taxonomic importance and may be considered in the identification and phylogenetic relationship of seeds or entire plants.

Ultrastructural features also help in identifying fragmentary parts of seed coats in both extant and extinct forms and assign them to the correct taxonomic unit. During the study of fragmentary plant parts from fossilized deposits, cuticles of leaf, sporangium and seeds of both angiosperms and gymnosperms are frequently recovered. Although there is a

wealth of information regarding leaf cuticle, identification of gymnosperm seed cuticles could rarely be done, as enough study of extant taxa has not been made. The present study on the seed coat of *Ephedra* may help in the formation of a database that can be utilized in the identification of fragmentary seed coat cuticles from fossilized deposits, compare with the extant ones and also help in tracing their affinity.

Based on ultrastructural features of the seed coat, an artificial key to the four studied species of *Ephedra* is suggested as follows:

wax present as minute platelets, epidermal cells elongated, fibrillar, coiled thread-like, anticlinal wall grooved, wall level not conspicuous, periclinal wall level flat, wall texture undulated, vasculature well developed

Ephedra foliata

wax uniformly present, amorphous in nature, epidermal cells rectangular, cuticle longitudinally striated, ridges and furrows closely placed, shallow, appearing wavy in nature, epidermal cells with oblique transverse wall, verrucose line indistinct, anticlinal wall flat, wall level not conspicuous, periclinal wall level slightly convex, wall texture almost smooth, vasculature well developed

Ephedra intermedia

wax present as minute platelets, cuticle with well-developed ridges and furrows, closely placed, densely reticulate, grooved, epidermal cells rhomboidal to polyhedral, depressed, transverse wall of the epidermal cell distinct, anticlinal wall grooved, anticlinal and periclinal wall level concave, wall texture serrated or dentate, vasculature not found, profuse solitary and aggregated starch grains present

Ephedra gerardiana

wax present, as minute platelets, cuticle ridged, usually exerted, epidermal cells elongated rectangular, transverse wall indistinct, anticlinal wall grooved, anticlinal wall and periclinal wall level convex, wavy, vasculature not found, profuse wax and starch grains (solitary and aggregated) present

Ephedra saxatilis

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Khejri, the king of Indian Thar desert is under phenophase change

Vegetation of Indian Thar region is ecologically important, though fragile. Any change in its composition and trait will ultimately impact the productivity and sustainability of the system and the region. This region has 682 species belonging to 352 genera and 87 families of flowering plants. Among them, *Prosopis cineraria*, locally called as Khejri or Jandi is an indigenous tree, which effectively stabilizes sand dunes and can withstand periodic burial[1]. It is believed to be the best suitable agroforestry species, due to its deep taproot system, positive allelopathy effect, soil fertility improvement and yield augmentation of understorey crops. Khejri offers nutritious supplementary food (pod flour, fruits, seed, etc.), top-feed (leaves and pods) and protection-cum-shelter for the benefit of humans and livestock during all the seasons, effectively during harsh periods. It offers lifeline to human beings during famine, as its powdered bark can be mixed with flour and made into cakes for consumption[2].

The population density of Indian Thar region is quite high compared with the global arid zone average of 6–8 persons/sq. km. It is projected that the population will increase from the present 22.5 million to 33.6 million and its density to 161 by 2025 (ref. 3). In agriculture, improved varieties, cultivation practice, plantation activities, protection measures and value addition have been adopted by farmers to meet the increasing population requirements. In fragile ecosystems like Thar Desert, pure cropping alone cannot