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SEED STRUCTURE IN RELATION TO THE TAXONOMY OF THE HIBISCEAE (*GOSSYPIUM, LEBRONNECIA* AND *THESPESIA*)

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Abstract

The general morphology and structure of the seeds of 7 genera and 8 species of Hibisceae have been studied. The paper discusses the relevance of seed structure to the tribal divisions of the family. In particular, the study has provided strong evidence for the inclusion of *Gossypium* and certain other genera in the tribe Gossypieae.

Introduction

As in most other natural groups, the differences between the genera and species of the Malvaceae are slight (Bentham & Hooker, 1862; Schumann, 1895; Edlin, 1935; Kearney, 1951; Borssum Waalkes, 1966; Fryxell, 1968). Studies with the light microscope of seed structure have been of importance to taxonomists. Vaughan, (1970) has dealt with the identification of commercial seeds and their products. As regards natural or phylogenetic classification, use too has been made of information concerning seed structure, although the number of investigations carried out have not been as many as those into the anatomy of angiosperm vegetative organs. The early work was well summarized by Netolitzky (1926) and in recent times, Corner (1976) has produced a most comprehensive account of dicotyledonous seed structure and development in relation to taxonomy. He emphasizes the importance of his approach in studies of phylogeny and natural classification and his publication surveys the available literature in a masterly fashion.

The scanning electron microscope (SEM) is being used widely to interpret the surface features of seeds and some reviews of the results obtained have recently appeared (Brisson & Peterson, 1976; Barthlott & Ehler, 1977). Transmission electron microscope (TEM) studies of seed structure have not been concerned primarily with taxonomy but have often dealt with applied problems related to seeds of economic importance (Vaughan, 1979).

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No work has been carried out on the seeds of *Lebronnecia* and *Thespesia.* Because of the great economic importance of several species of *Gossypium,* much research, involving the light microscope, has been carried out into the structure and development of the seeds of the species (Vaughan, 1970; Joshi, Wadhwani & Johri, 1967; Ramchandani, Joshi & Pundir, 1966; Reeves & Valle, 1932; Reeves, 1935). The cotton seed develops from an anatropous, bitegmic, crassinucellate ovule. The outer epidermis of the seed consists of thick-walled cells with dark contents and presents a rather convoluted appearance in surface view. Scattered stomata are found in the epidermis. The hairs, which are naturally unbranched outgrowths of single epidermal cells, are of two sorts: (i) long hairs (staple lint, 0.5–50 mm long, arising before fertilization or in the ensuing 1–6 days) (ii) short hairs (linters or fuzz, 1–5 mm long, developing 7–12 days after fertilization). Commercial 'ginning' removes the lint but the fuzz remains on the seed and shows a varying distribution according to species and variety. Below the epidermis are two or three layers (different species show some variation as regards number of layers) of parenchyma cells with brown contents and in this zone is embedded the raphe vascular tissue. A clear layer is found with the pigmented zone, some cells of which contain small calcium oxalate crystals. All the cell layers so far described develop from the outer integument of the ovule.

The outermost layer of the inner integument forms the highly characteristic palisade of Malpighian cells, 150–240 μm long. These cells are a special feature of the seed coats of the Malvaceae, also the Bombacaceae. Different parts of the wall of the palisade cell give either lignin or cellulose reactions, possibly according to species and variety. The cell lumen is only obvious in the upper portion of the palisade cell and a 'light line' can usually be seen. The remainder of the testa consists of rather flattened cells, many of which contain tannins. A layer of cells with curiously pitted walls, the 'fringe layer', forms the innermost layer of the testa.

The endosperm is reduced, often to one layer of cells with minute aleurone grains. The outer epidermis of the folded cotyledons may show stomata and oval jointed hairs. Most of the cotyledon cells are isodiametric parenchyma, with oil drops, occasional cluster crystals of calcium oxalate and aleurone grains as ergastic inclusions. Some starch may also be present. Procambium is also to be found in the cotyledon tissue and there is a single layer of palisade cells immediately within the inner epidermis. An outstanding feature of the embryo is the presence of gossypol (a phenolic compound) cavities, up to 120 μm in diameter.

A number of electron microscope studies have been carried out into the seed structure of *Gossypium.* A SEM study of the development of the seed has been produced by Beasley (1975). Some TEM accounts of the embryo cell contents have been written (Yatsu, 1965; Vix, Gardener, Lambou & Rollins, 1972; Tauma-Touchan, 1977). The

great commercial importance of the seed hairs has given rise to a considerable number of SEM and TEM investigations of the fibre structure (DeGruy, Carra & Goynes, 1973; Stewart, 1975; Ramsay & Berlin, 1976a and b). Changes in the anatomy of the *Gossypium* seed coat caused by lucerne saponins have been reported by Marchaim, Werker & Thomas (1974).

The present investigation is a study of the seed structure of 3 genera and 8 species of Hibisceae, using light microscopy and the scanning electron microscope. The structural variation described is related to the taxonomy of the tribe.

Material and Methods

Seed samples were obtained from various sources. Some samples were received with voucher numbers. In the case of unauthenticated material, seeds were grown at the Chelsea Physic Garden. When possible, the resulting plants were identified against dried specimens in the herbarium of the Natural History Museum (London). In a number of cases, it was not possible to authenticate material because either the seed did not germinate or plants grown at the Chelsea Physic Garden did not produce flowers. Herbarium sheets were prepared of all material grown.

For each accession, five seeds were taken as a representative sample. Written descriptions of the external features of the seed were produced for each species. Also, drawings were made of the external appearance and the appearance in transverse section of the seed as seen under the dissecting microscope (X30).

As regards anatomical investigations, prior to sectioning, seeds were soaked overnight in cold water to improve the softening process. Transverse sections of the seeds were cut with a Reichert sliding microtome, using a wedge shaped knife set at a cutting angle of 45° and an inclined angle of 13°. The thickness of sections was set at 20 μm. Some seeds were cut directly but in the case of small seeds, these were held between two pieces of carrot. While cutting the sections, the seeds and knife were flooded with 70% ethyl alcohol and the sections then transferred to tap water.

The following histochemical tests were applied to the sections: sudan blue (oil); 5% ferric chloride (tannins); 1% iodine (starch); chloro-zinc-iodine (cellulose); bromophenol blue (protein); phloroglucinol and concentrated HCl (lignin).

The appearance of the hairs on the outside of the testa was studied in surface view by clearing softened pieces of the seed coat in chloral hydrate. Permanent slides were prepared of sections stained with Delafield's haemotoxylin according to the procedure of Vaughan (1960). Photomicrographs were prepared with a Zeies photomicroscope.

Stereoscan pictures were taken at the Jodrell Laboratory (Royal Botanic Gardens, Kew). A Jeol SI Scanning Electron Microscope, operated at 10 Kv and tilt 45, was employed. Dry seeds were directly attached to the mounting stubs with Durafix glue and were coated with gold. The stubs were inserted into the vacuum chamber, evacuation was rapidly accompushed and photographs were taken immediately after location of the desired area and determination of the most useful magnification.

The following abbreviations have been used for the seed characters:

GM: General morphology
L: Seed length
C: Colour; BL: Black; BR: Brown; LB: Light Brown: GY: Golden Yellow.
S: Shape; P: Pear-shaped.
FA: Funicle absent.
CO: Cotyledons;
MF: Much folded.
H: Hairs: The minimum and maximum length is given for hairs, together with the average (a).
T: Testa;
OE: Outer epidermis, developed from outer epidermis of outer integument.
OM: Mesophyll of outer integument.
IE: Inner epidermis of outer integument.
PL: Palisade layer, developed from outer epidermis of inner integument.
IM: Mesophyll of inner integument.
FL: Fringe layer, developed from inner epidermis of inner integument.
GC: Gossypol cavities in embryo.

Results

The general morphology and the internal structure of the seed of each species showed variation with regard to seed length, colour and shape. Three shapes have been recognized: cuneiform (wedge-shaped); compressed reniform with one end narrow; pear-shaped. The funicle may be persistent or absent. In section, the cotyledons are seen to be slightly folded or much folded.

The hairs on the seed surface show variation of distribution and structure. Various regions of the testa show differences in microscopic structure. These include: the outer epidermis (developed from the outer epidermis of the outer integument); the mesophyll of the outer integument; the inner epidermis of the outer integument; the palisade layer (outer epidermis of inner integument); the fringe layer (inner epidermis of innter integument).

A description of the seed characters for each species is as follows:

Gossypium arboreum L. Accession: K/85

GM: L, lcm; C, BL; S, P; FA; CO, MF.

H: Form a thick mat over the testa; unicellular; long hairs (lint), 19.50–21.55, a. 20.525 mm; short hairs (fuzz), 7.50–10.45, a. 9.00 mm.

T: OE, thick-walled cells with dark contents; OM, 2–7 layers of cells with tannin contents; IE, 1 layer of colourless thick-walled cells, some with calcium oxalate crystals; PL, 245 μm, lumen in middle of cell; IM, 3–4 layers of cells with tannin contents; FL, present; GC, present Figs. 1A, B; 2A; 4.

Gossypium barbadense L. Accessions: K/52, K/164

GM. L, lcm; C, BL; S, P: FA; CO, MF.

H: Form a thick mat over the testa; unicellular; long hairs (lint), 34.0–44.50, a. 39.25 mm, short hairs (fuzz), 900 μm - 2.10 mm, a. 1.50 mm.

T: OE, thick-walled cells with dark contents; OM, 3–9 layers of cells with tannin contents; IE, 1 or 2 layers of colourless thick-walled cells, some with calcium oxalate crystals; PL, 205 μm, lumen in middle of cell; IM, 2–3 layers of cells with tannin contents; FL, present. GC, present. Fig. 2B.

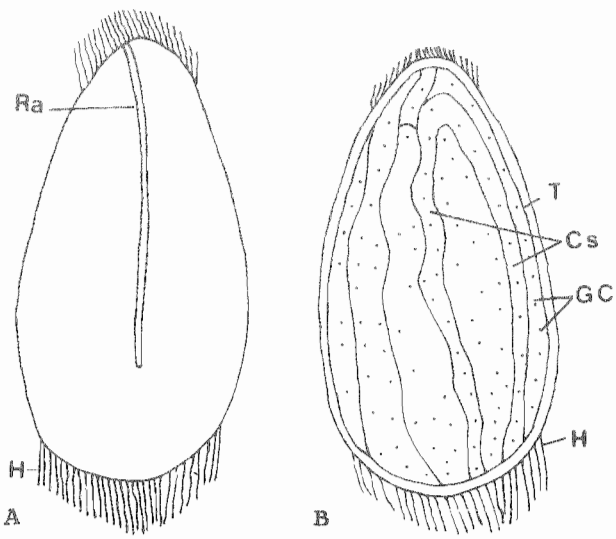

Fig. 1. *Gossypium arboreum* L.
A. Whole seed showing raphe (Ra), X 30
B. Section through seed showing foldings of cotyledons, X 30.
Cs, Cotyledons; GC, Gossypol cavities in embryo; H, Hairs; Ra, Raphe; T, Tests.

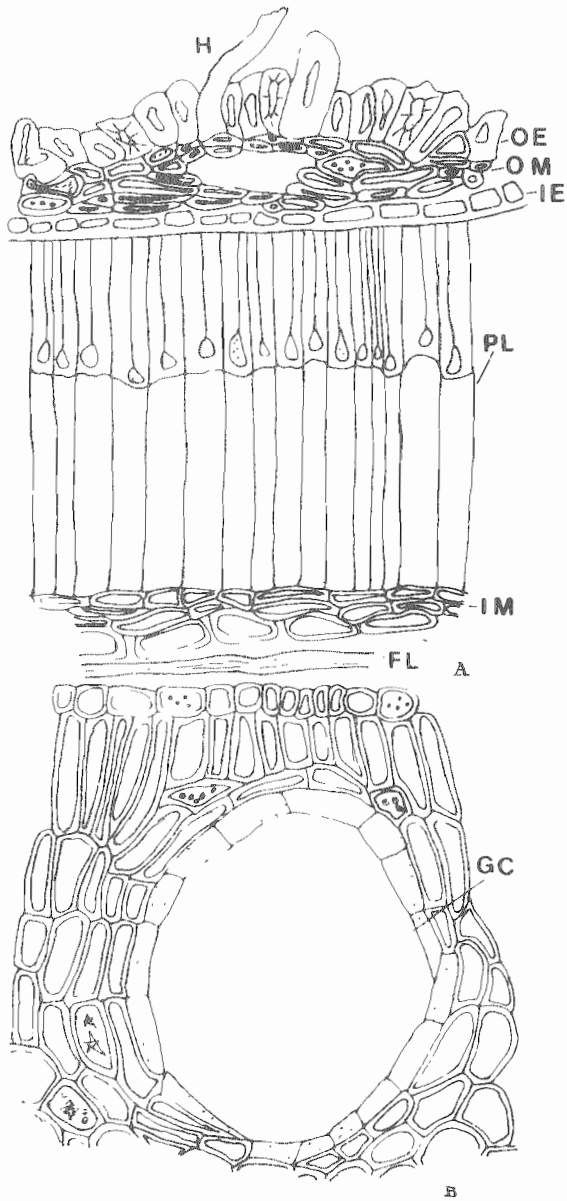

Fig. 2.A. *Gossypium arboreum* L. Transverse section of seed coat, X 470.
B. *Gossypium barbadense* L. Transverse section of embryo, X 470.
FL, Fringe layer developed from inner epidermis of inner integument; GC, Gossypol cavities in embryo; H, Hairs; IE, Inner epidermis of outer integument; IM, Mesophyll of inner integument; OE, Outer epidermis, developed from outer epidermis of outer integument; OM, Mesophyll of outer integument; PL, Palisade layer, developed from outer epidermis of inner integument.

Gossypium herbaceum **L.** Accessions: K/86, K/165.

GM: L, Icm; C, BL; S, P; FA; CO, MF.
H: Form a thick mat over the testa; unicellular; long hairs (lint), 11.50–20.50, a. 16.00 mm; short hairs (fuzz), 450 μm - 1.05 mm, a. 750 μm.
T: OE, thick-walled cells with dark contents; OM, 2–5 layers of cells with tannin contents; IE, 1 layer of colourless thick-walled cells, some with calcium oxalate crystals; PL, 265 μm, lumen in middle of cell; IM, 1–4 layers of cells with tannin contents; FL, present. GC, present.

Gossypium hirsutum **L.** Accession: K/166

GM: L, Icm, C, BL; S, P; FA; CO, MF.
H: Form a thick mat over the testa; unicellular; long hairs (lint), 1.00–4.05, a. 2.52mm; short hairs (fuzz), 200–750, a. 475 μm.
T: OE, thick-walled cells with dark contents; OM, 2–8 layers of cells with tannin contents; IE, 1 layer of colourless thick-walled cells, some with calcium oxalate crystals; PL, 190 μm, lumen in middle of cell; IM, 2–5 layers of cells with tannin contents; FL, present. GC, present.

Gossypium wightianum **Tod.** Accession: K/54.

GM: L, Icm; C, BL; S, P; FA; CO, MF.
H: Form a thick mat over the testa; Uni-bi-and multicellular, pigmented; long hairs (lint), 550 μm-2.00 mm, a. 1.27 mm; short hairs (fuzz), 125-475, a. 300 μm.
T: OE, thick-walled cells with dark contents; OM, 4–7 layers of cells with tannin contents; IE, 1 or 2 layers of colourless thick-walled cells, some with calcium oxalate crystals; PL, 210 μm, lumen in middle of cell; IM, 1–6 layers of cells with tannin contents; FL, present. GC, present. Fig. 3

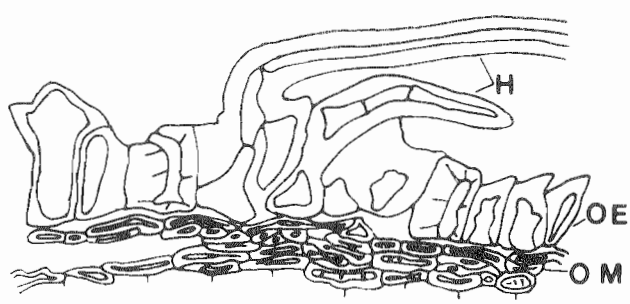

Fig. 3. *Gossypium wightianum* Tod.
Transverse section of seed coat, X 470.
H, Hairs; OE, Outer epidermis, developed from outer edpiermis of outer integument; OM, Mesophyll of outer integument.

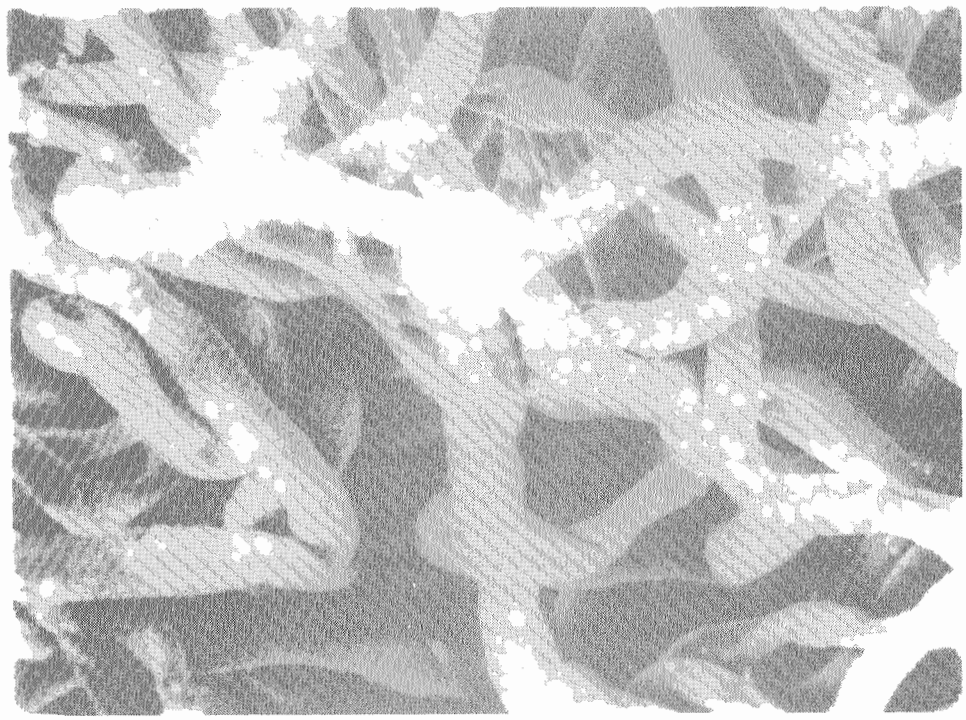

Fig. 4 *Gossypium arboreum* L.
SEM, showing hairs on seed surface X 3000.

Lebronnecia kokioides **Fosberg.** Accession: K/274

GM: L, 5–15 mm; C, BR; S, P; FA; CO, MF

H: Form a thick mat over the testa; unicellular, pointed; 130 μm - 8.75 mm, a. 4.50 mm.

T: OE, thick-walled cells with dark contents; OM, 2–7 layers of cells with tannin contents; IE, 1 or 2 layers of colourless thick-walled cells, some with calcium oxalate crystals; PL, 230 μm, lumen in upper 1/3 of cell; IM, 5–9 layers of cells with tannin contents; FL, not present. GC, present.

Thespesia populanea **(L.) Soland. ex Correa**
(= *Hibiscus populneus* L.) Accession. K/230

GM: L, 1cm; LB; S, P; FA; CO, MF.

H: Unicellular, pointed; 140–500, a. 320 μm; at both ends, very large hairs present, a. 1.20 mm.

T: OE, thick-walled cells with dark contents; OM, 2–7 layers of cells with tannin contents; IE, 1 or 2 layers of colourless thick-walled cells; some with calcium oxalate crystals; PL, 305 μm, lumen almost throughout cell; IM, 1–11 layers of cells with tannin contents; FL, present. GC, present.

***Thespesia populneoides* (Roxb.) Kostel.** Accession: K/231

GM: L, 7–10mm; C, GY; S, P; FA; CO, MF.
H: Unicellular, club-shaped; 165–595, a. 380 μm.
T: OE, thick-walled cells with dark contents; OM, 2–7 layers of cells with tannin contents; IE, 1 or 2 layers of colourless thick-walled cells, some with calcium oxalate crystals; PL, 330 μm, lumen in upper 1/13 of cell; IM, 1–4 layers of cells with tannin contents; FL, present. GC, present.

Discussion

In the present investigation, the seed structure of 8 species of Hibisceae has been described. However, the taxa investigated have not presented any new marked variations in structure and, as is generally accepted, the seed structure supports the concept of the family as a natural unit. Nevertheless, the genera *Gossypium, Thespesia* and *Lebronnecia* can be distinguished in that (i) the seed hairs are very long and persistent (ii) the walls of the outer epidermal cells are thick (iii) an outer mesophyll limited by an inner layer of cells containing calcium oxalate crystals is present (iv) gossypol cavities are present in the cotyledons. In the present investigation, the seed hairs have been described as club-shaped and pointed.

The division of the family into various tribes were based on various characters of the flower and fruit (Bentham & Hooker, 1862; Schumann, 1895; Kearney, 1951; Hutchinson, 1967). Seed structure, as described in this investigation, does not in general provide information for the division of the family into tribes. However, the seed structure of *Gossypium, Thespesia* and *Lebronnecia* is distinct. Fryxell (1968), on the basis of embryo structure and the presence of gossypol glands, removed these genera (together with *Cienfuegosia, Hempia, Kokia, Cephalohibiscus* and *Gossypioides*) from the Hibisceae into a resurrected tribe Gossypieae. The anatomical studies now presented support this separation and it would be interesting to investigate the seed structure of *Cienfuegosia, Hempia, Kokia, Cephalohibiscus* and *Gossypioides* confirm the thesis of Fryxell (1968).

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