Seed development in *Trewia nudiflora* Linn.

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Development of seed coat and structure of mature seed of *Trewia nudiflora* Linn. have been investigated. Ovary is two to five chambered and each locule has a single ovule which is anatropous, crassinucellate and bitegmic. Development of the female gametophyte is of the *Polygonum*-type. Endosperm development is of the Nuclear-type and mature embryo occupies full length of a seed. Seed coat is formed by both integuments. The outer integument forms fleshy testa. Outer epidermis of the inner integument forms radially elongated, obliquely oriented macrosclereid layer. SEM studies on spermoderm pattern have been done.

Key-words – Angiosperm, Seed development, *Trewia nudiflora*, Euphorbiaceae.

INTRODUCTION

BANERJI and Dutt (1944), and Pal and Chopra (1987) investigated the development of female gametophyte in *Trewia nudiflora* and gave a brief account of ovule and obturator. Rao and Rao (1974) reported presence of druses in the integuments of this taxon. More details of the development of seed are being furnished.

Trewia nudiflora is a large deciduous tree characterized by solitary female flowers, absence of petals, 3 to 5 styles which are connate at the basal side and long papillose stigma. This species is found in Sri Lanka, India, Pakistan and Thailand. In India, *Trewia nudiflora* is distributed in forests of Dehradun, Saharanpur extending eastwards along the sub-Himalayan forest tracts of Ruhelkhand, North-Avadh and Gorakhpur. It grows profusely in Assam and Khasi Hills (Hooker, 1872).

MATERIAL AND METHOD

Flowers and fruits of *Trewia nudiflora* at different developing stages were collected from the National Botanical Research Institute, Lucknow. Fresh seeds were fixed in FAA and subsequently preserved in 70% ethanol. Usual methods of dehydration in tertiary butyl alcohol series and embedding in paraffin wax were employed (Sass, 1958). Serial microtome sections, cut between 10-15 µm thickness, were stained in safranin - fast-green combination.

OBSERVATION

In *Trewia nudiflora* the male and female flowers are borne on separate trees. In the gardens of National Botanical Research Institute, Lucknow only female trees are present. The female flowers are green, axillary, solitary or in groups of two to three; their lower surface is covered with cottony wool. The calyx, generally five-toothed, closely surrounds the ovary till it drops at maturity.

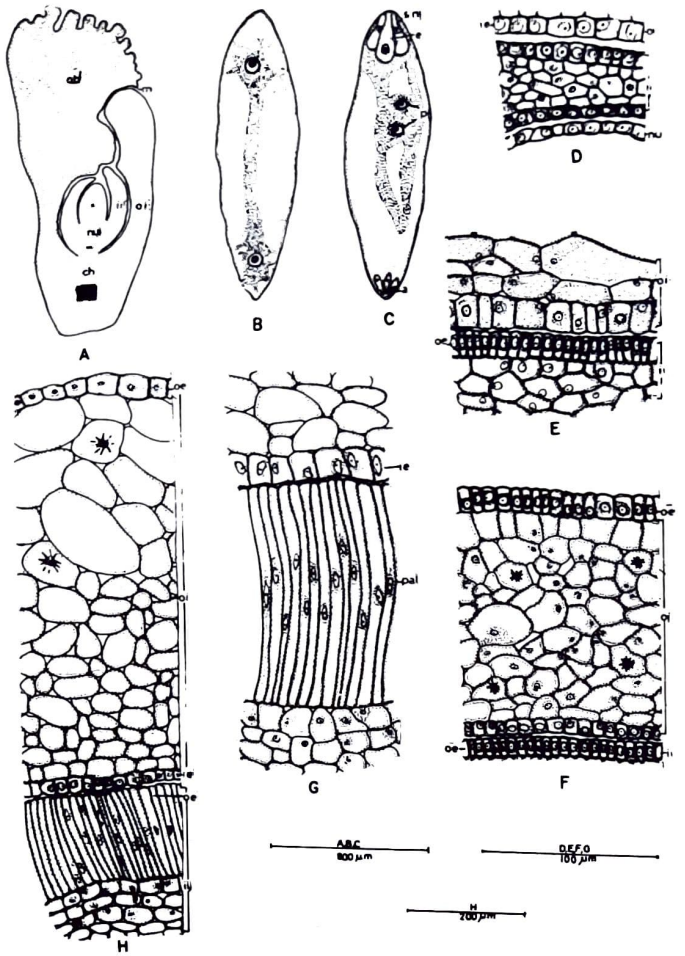
The ovule is anatropous, crassinucellate and bitegmic (Text-fig. A; Pl. 1, fig. 1). Both the integuments are massive and form a long and narrow micropyle. The pattern of ovular supply is similar to that as described by Singh (1962).

The obturator arises from placenta just above ovular attachment and tends to cover the micropyle (Pl. 1, figs. 1, 2; Pl. 3, fig. 1). It is composed of parenchymatous cells. After fertilization, it becomes tanniniferous and eventually degenerates. Different stages of megasporogenesis and megagametogenesis (Pl.1, fig. 3; Text-figs B,C) are similar as observed by Pal and Chopra (1987).

The inner integument possesses a broad base and its inner margin arises at a much higher level than the outer one. The outer integument is thicker than the inner and completely overgrows the latter during ovule development.

Seed development

Normally all the ovules in an ovary develop into mature seeds and during the process considerable changes occur in different parts of a fertilized ovule. The structure of endosperm, nucellus, embryo have been reported earlier (Banerji & Dutt, 1944; Pal & Chopra, 1987).



Text-figures A-H. *Trewia nudiflora*-Development of female gametophyte and seed: **A.** A part of longi-section of the ovary showing anatropous ovule and obturator. **B.** Longi-section of ovule at the stage of 2-nucleate embryo sac. **C.** Longi-section of a ovule at the stage of organized female gametophyte showing egg aparatus, two polars and three antipodals. **D.** A part of longi-section of seed coat at the stage of megaspore mother cell showing integumentary layers and the nucellar epidermis. **E.** A part of longi-section of seed coat at the stage of female gametophyte. **F.** A part of longi-section of mature seed coat showing outer integument and outer epidermis of inner integument. **G.** Same as in Text-fig. E, at young globular embryo stage. **H.** A part of seed coat in mature seed showing characteristic sclerenchymatous cells of inner integument.

The nucellus is quite massive. At the chalazal end, a few cells lying directly below the embryo sac (almost in the centre of the nucellar tissue) become differentiated by their dense cytoplasm and compact arrangement (Pl. 3, fig.4). In due course of time, these cells divide and proliferate actively in the chalazal area, thus responsible for considerable enlargement of the seed (Pl.3, figs 2,3). Eventually the cells of hypostase get thickened and surrounded by the ring of vascular strands which are ramification of the main chalazal supply (Pl.3, fig.1). As the embryo matures, chalazal region becomes broader, accompanied with the extensive growth of chalazal tissue in this region.

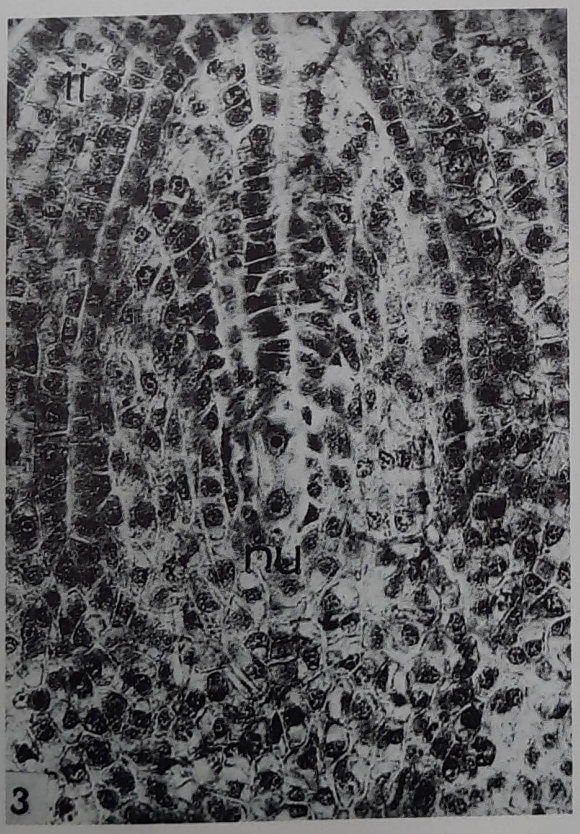
Inner integument - Outer epidermal cells of the inner integument get radially elongated giving a palisade-like appearance (Text-fig. D,E,F; Pl.1, fig.4; Pl.3, fig. 3). In later stages, this elongation also extends to lower part and the cells become some what obliquely bent (Text-fig. G,H). Later, their walls get sclerified and show thickenings. Each macrosclereid is broader towards the periphery of the seed and narrower towards the endosperm; these cells form the characteristic sclerenchymatous zone (Text-fig. H). The cells of inner epidermis elongates tangentially and gradually lose their contents. The hypodermal cells are small in size and ultimately get crushed. During further development of the seed most of integumentary cells lying below the sclerenchymatous layer persist as a membranous sheath.

Outer integument - Outer epidermal cells of the outer integument undergo some tangential elongation. The mature seed derives its dark brown colour, often stippled with light-brown patches, from the contents of outer layer. The inner epidermal cells become columnar and compact (Text-fig. G). The remaining cells elongate tangentially and lose their contents (Text-fig. H). Shinning crystals of calcium oxalate are frequently distributed in both integuments even prior to fertilization.

Mature seed coat - Both the integuments take part in the formation of seed coat, which is differentiated into testa and tegmen. The tegmen is formed of two portions - the outer one being derived from the sclerenchymatous layer and the inner one by remaining layers of the inner integument in continuation with the crushed cells of chalaza. The vascular supply of chalaza persists even in a somewhat crushed condition. A massive endosperm and a well-developed embryo of the Spatulate-type are surrounded by the seed coat.

As viewed under SEM, the seed surface shows ridges arranged in a somewhat reticulate fashion. Under different magnification the cells are polygonal showing ridges and uneven foldings (Pl.2, figs 3,4,5). However, rest of the surface appears to be smooth. The margins of the cells are distinct. In mature seed, small irregular cracks are visible which may be due to shrinkage and these may be useful in imbibing water at the time of germination (Pl.2, fig.2).

Seed -The dorsal surface of seed is slightly convex whereas the ventral surface has a projecting ridge in the centre formed by the remains of the raphe. The mature seeds are dark-brown, more or less oval in outline, 7 x 7 mm in size and excarunculate. On the surface of the seed the ridges are arranged in a reticulate manner, which may be formed because of an unequal elongation of the sclerenchymatous layer.



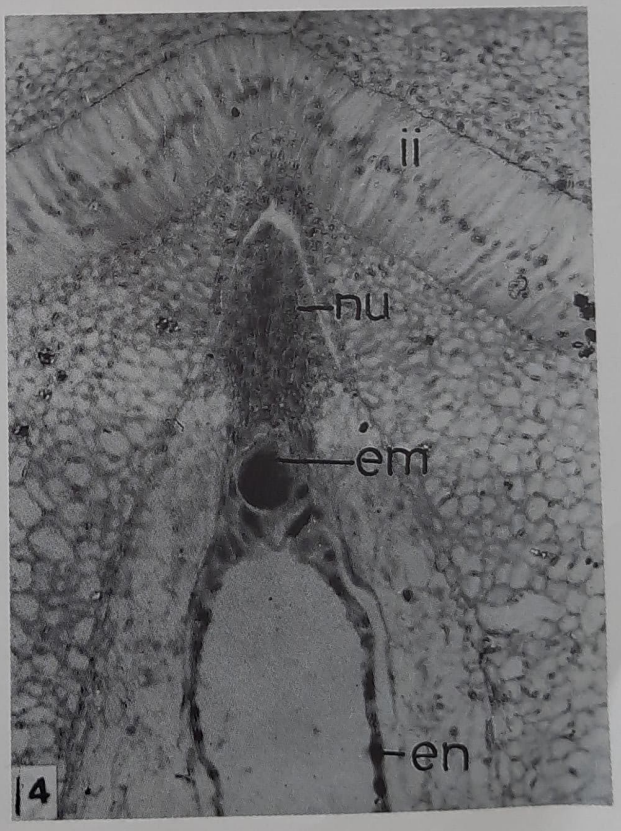


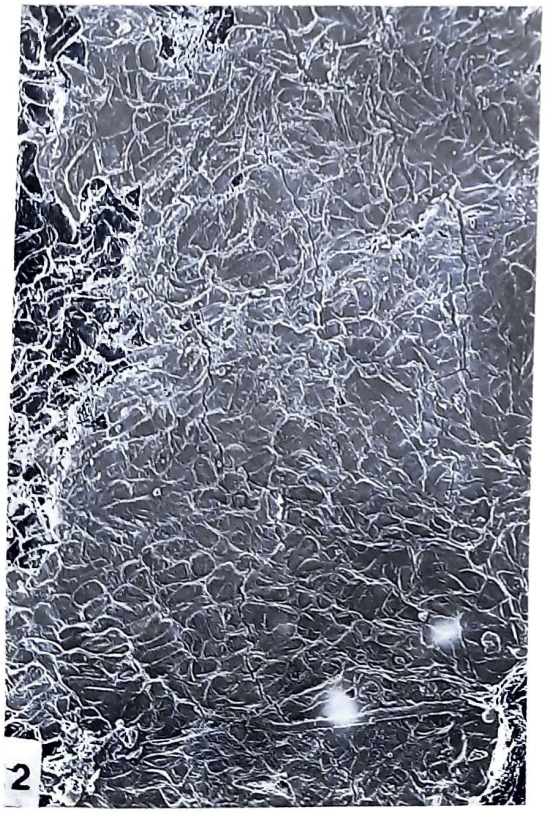
Plate 1

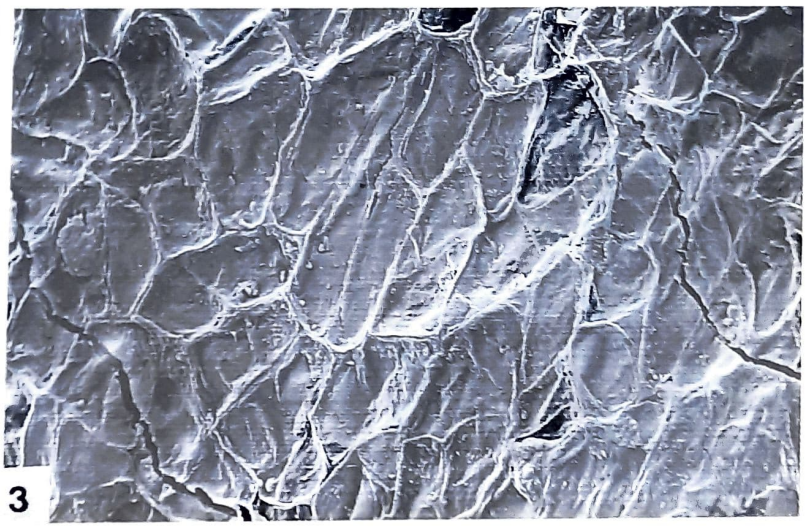
Trewia nudiflora - Stages of seed development.

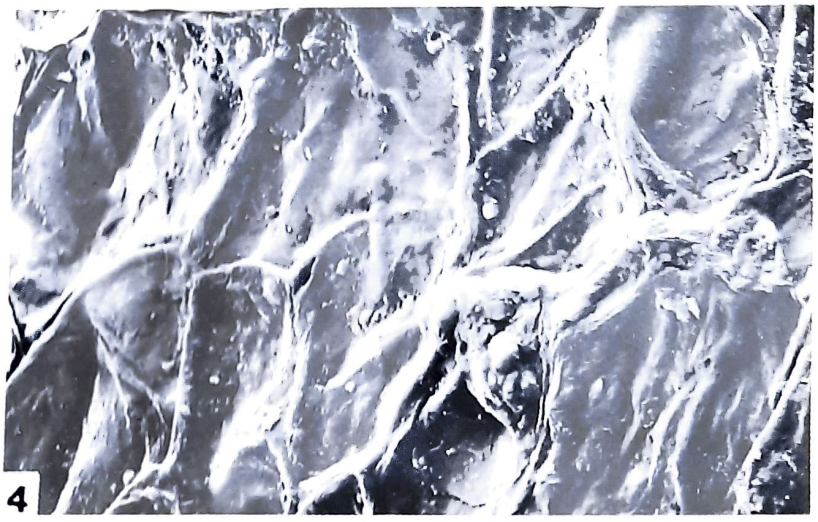
1,2. A portion of longi-section of ovary showing anatropous ovule and obturator, A x 35; B x 60.

3. A part of ovule showing 2-nucleate embryo sac, x 370.

4. A part of longi-section of seed at the globular embryo stage showing endosperm, nucellus and inner epidermis of the inner integument, x 100.







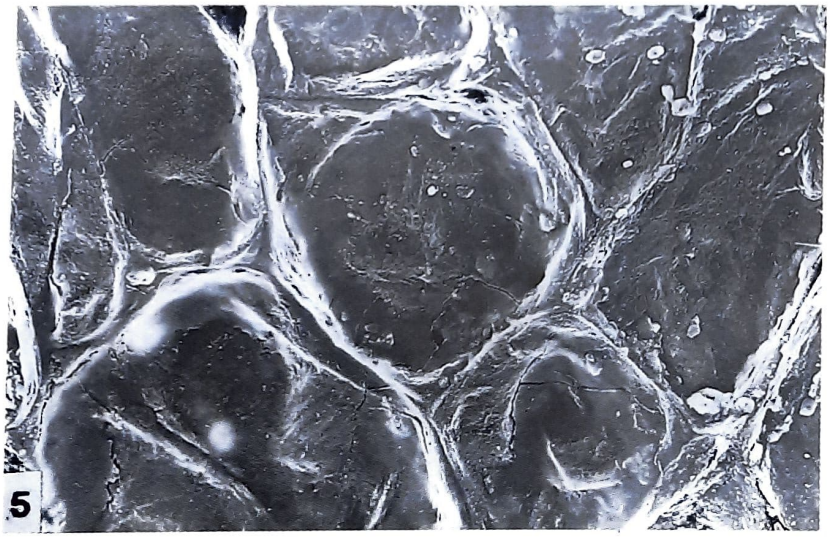
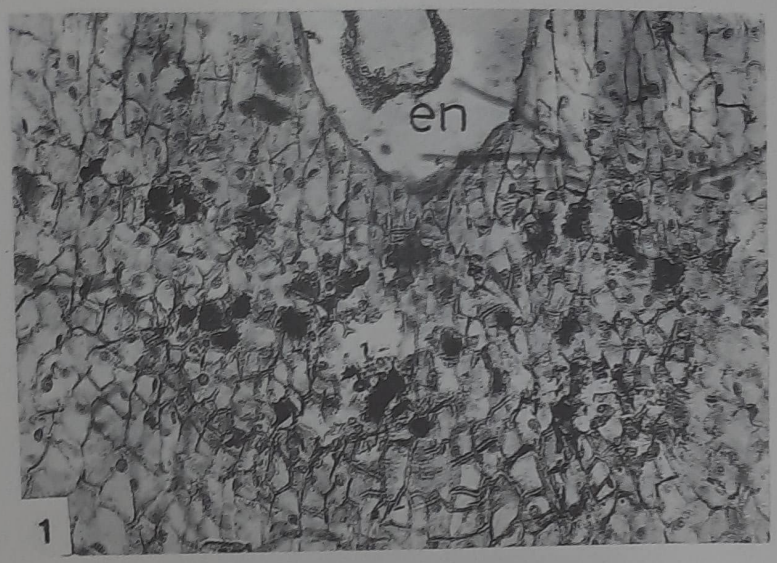


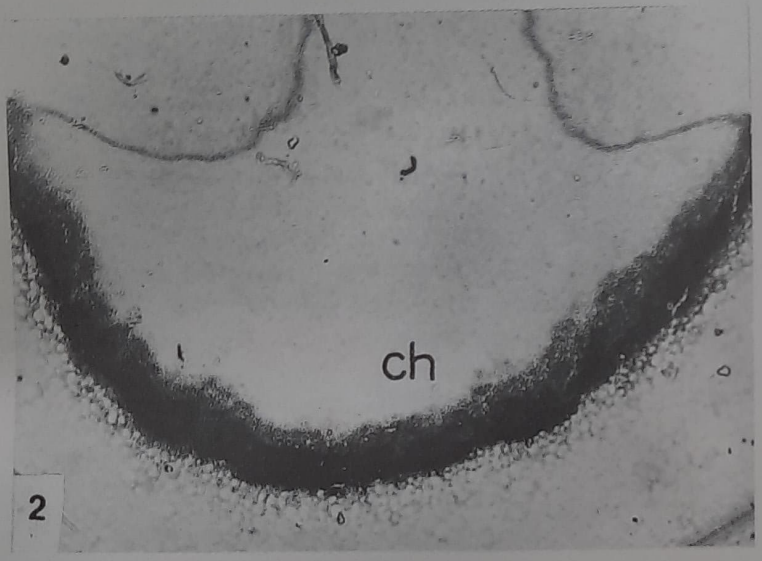
Plate 2

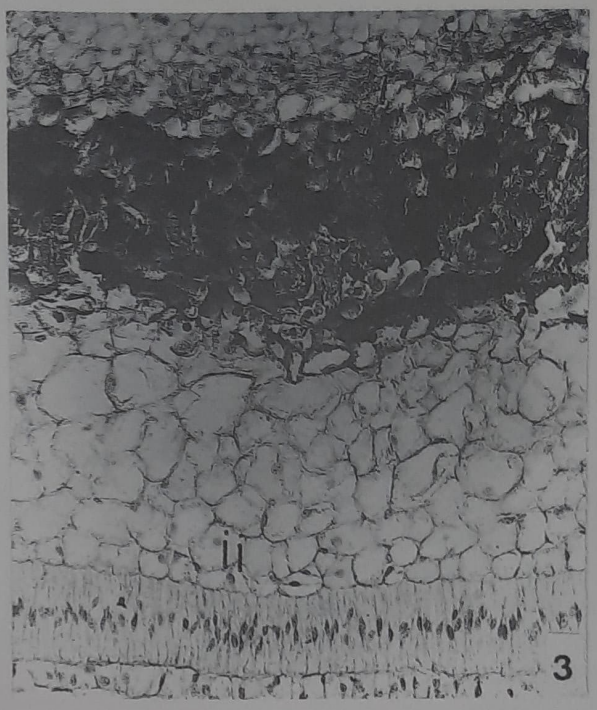
Trewia nudiflora-Morphology of obturator and spermoderm.

1. Longi-section of ovule showing an extensive obturator, x 75.

2-5. Dry and mature seed coat showing cracks and raised polygonal walls, 2 x 75; 3 x 300; 4 x 800; 5 x 825.







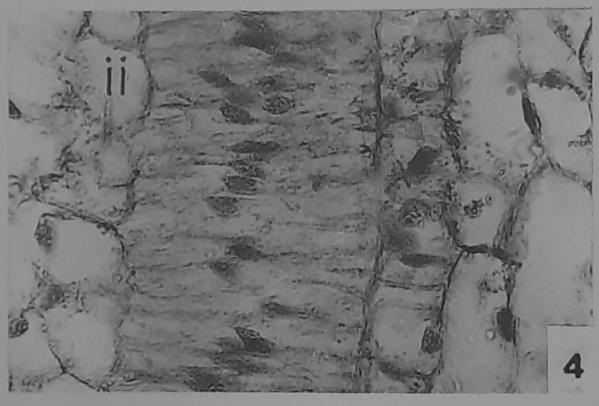


Plate 3

1. A part of longi-section of seed showing hypostase, x 260.
2. A part of longi-section of seed showing chalazal part surrounded by a ring of vascular strands, x 20.
3. A part of longi-section of seed showing palisade-like inner epidermal cells of the inner integument with characteristic thickenings, x 500.
4. A part of longi-section of seed showing deeply-stained cells of vascular strands and inner epidermis of the inner integument, x 170.

The embryo occupies full length of seed and shows a well-marked root cap. The vascular supply branches freely in flat, broad cotyledons.

DISCUSSION

The obturator in *Trewia nudiflora* is of placental origin as in other cases of Euphorbiaceae and it forms a number of lobes. After fertilization the obturator dries out gradually. It is assumed that obturator guides the pollen tube to the embryo sac and provides nourishment.

Differentiation of hypostase is marked at the base of the organized embryo sac even before fertilization in *Trewia*. Considerable tissue intervenes between the hypostase and the point at which the inner epidermis of the inner integument diverges from the nucellus. Extensively grown chalaza has also been recorded in *Trewia* as found in *Melanthesa rhamnoides* (Singh, 1968).

According to Maheshwari (1950), hypostase acts as a barrier to prevent excessive expansion of the megagametophyte. Boesewinkel and Bouman (1984) interpreted hypostase as a kind of glandular tissue secreting hormones eventually required for the growth of embryo sac, as a tissue connecting the chalazal bundle with the embryo sac and thus facilitating transport of food materials, as a tissue regulating the water economy of dormant seed, or as a barrier tissue preventing the extension of embryo sac. The cells of hypostase may

accumulate carbohydrate and protein substances in the vacuoles as well as in the cytoplasm. In some way it is related to the translocation of nutrients into the megagametophyte and finally the embryo. It may function secondarily as a storage tissue. Any definite physiological role of hypostase is difficult to assign (Bhatnagar & Johri, 1972).

Endosperm cells are filled with food reserves particularly oil. It occupies the major volume of the mature seed, containing fat globules and crystals of calcium oxalate.

In *Trewia nudiflora*, the testa of mature seed coat is formed by entire outer integument and the chalazal part lying in its continuation. Testa is fleshy and the mechanical layer of tegmen is formed by the outer epidermis of inner integument. It is characterized by thick-walled macrosclereids. The plane of elongation of sclereids is in radial direction in relation to the long axis of seed which is a characteristic feature of the members of Crotonoideae (Singh, 1985).

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