STRUCTURE AND DEVELOPMENT OF SEEDS IN *PUTRANJIVA ROXBURGHII* WALL.

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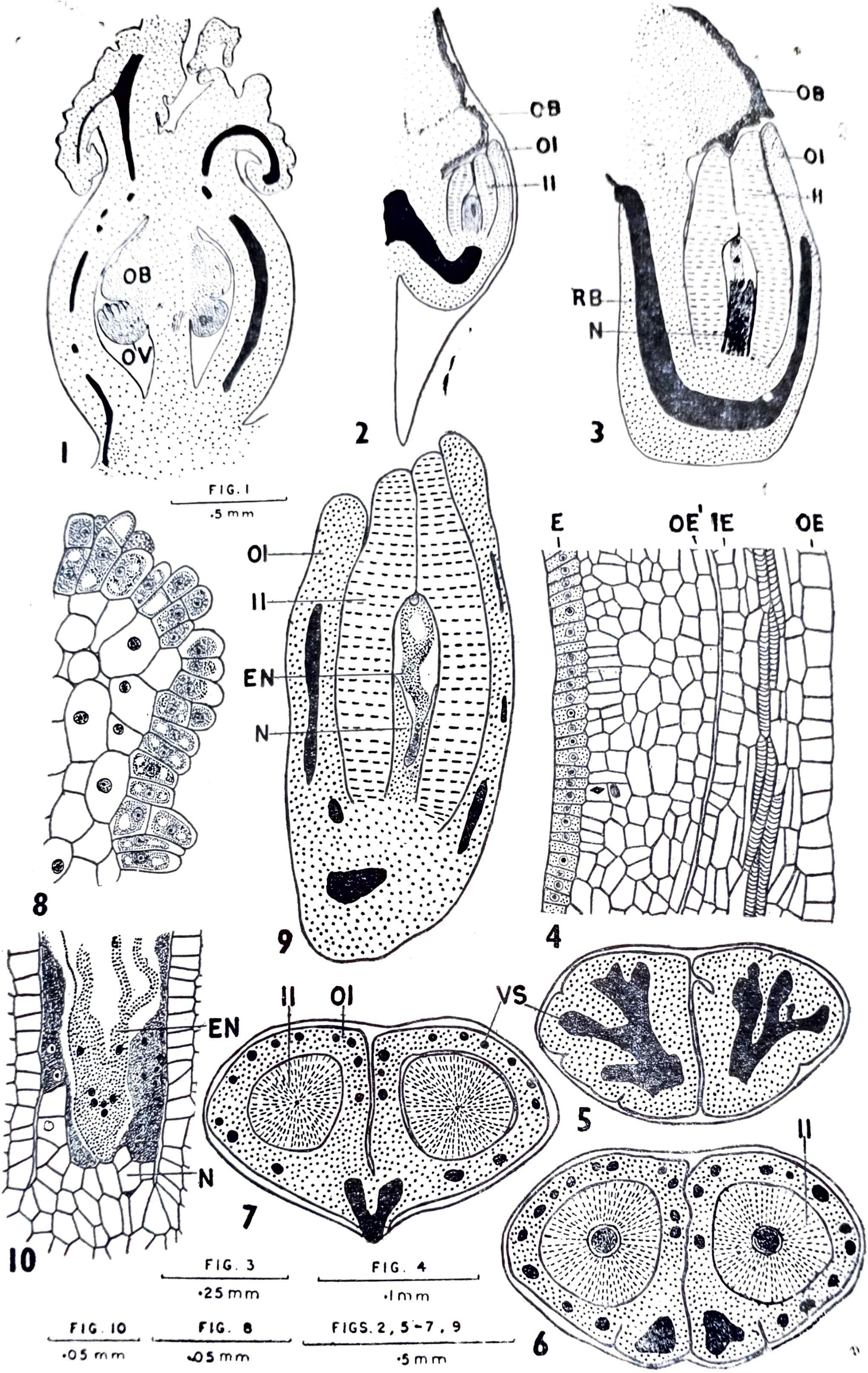
INTRODUCTION

Putranjiva roxburghii belongs to the family Euphorbiaceae, sub-family Phyllanthoideae, tribe Phyllantheae, and sub-tribe Glochidiinae (Pax and Hoffmann, 1931). Certain aspects of embryology of this species have been described by Banerji and Dutt (1944) and Thathachar (1953). Their accounts, however, do not describe certain features of the ovule and the development of the seed, especially the seed coat which appears to the author to be of considerable interest for the drupaceous fruit of this species is provided with a thick stony endocarp. The present article, therefore, deals with the development of the seed laying more emphasis on the seed coat.

MATERIAL AND METHODS

Flowers and fruits of *Putranjiva roxburghii* were collected locally as well as from Taj Gardens, Agra, and fixed in Formalin-acetic-alcohol. They were dehydrated and cleared through alcohol-xylene, as well as *ter.* butyl alcohol series and embedded in paraffin wax in the usual way. Serial sections, between 10 to 20 microns thick, were stained with Heidenhains-iron-haematoxylin and safranin-fast green combinations. Mature seeds were also hand-sectioned. Maceration of the sclereids of the seed coat was done according to Jeffrey's method (Johansen, 1940).



FIGS. 1–10. Fig. 1. L.s. gynoecium showing young ovules and obturator Fig. 2. L.s. ovule and obturator at the megaspore tetrad stage. Fig. 3. L.s. ovule at the fully organised female gametophyte stage. Note that the micropyle

is completely closed and nucellus persisting only at the base of the embryo-sac. Fig. 4. L.s. part of integuments at the fully organised female gametophyte stage. Figs. 5-7. T.s. at different levels of the ovule. Note the imperceptible micropyle in Fig. 7. Fig. 8. L.s. part of obturator. Fig. 9. L.s. ovule after fertilisation. Note the penetration of free nuclear endosperm in the nucellar column. Fig. 10. L.s. part of ovule showing the nucellar column and the free nuclear endosperm. Note the degenerating cells of the nucellus.

E, tapetum; *EN*, endosperm; *IE*, inner epidermis of outer integument; *II*, inner integument; *N*, nucellus; *OB*, obturator; *OE*, outer epidermis of outer integument; *OE'*, outer epidermis of inner integument; *OI*, outer integument; *OV*, ovule; *RB*, raphe bundle; *VS*, vascular supply.

Observations

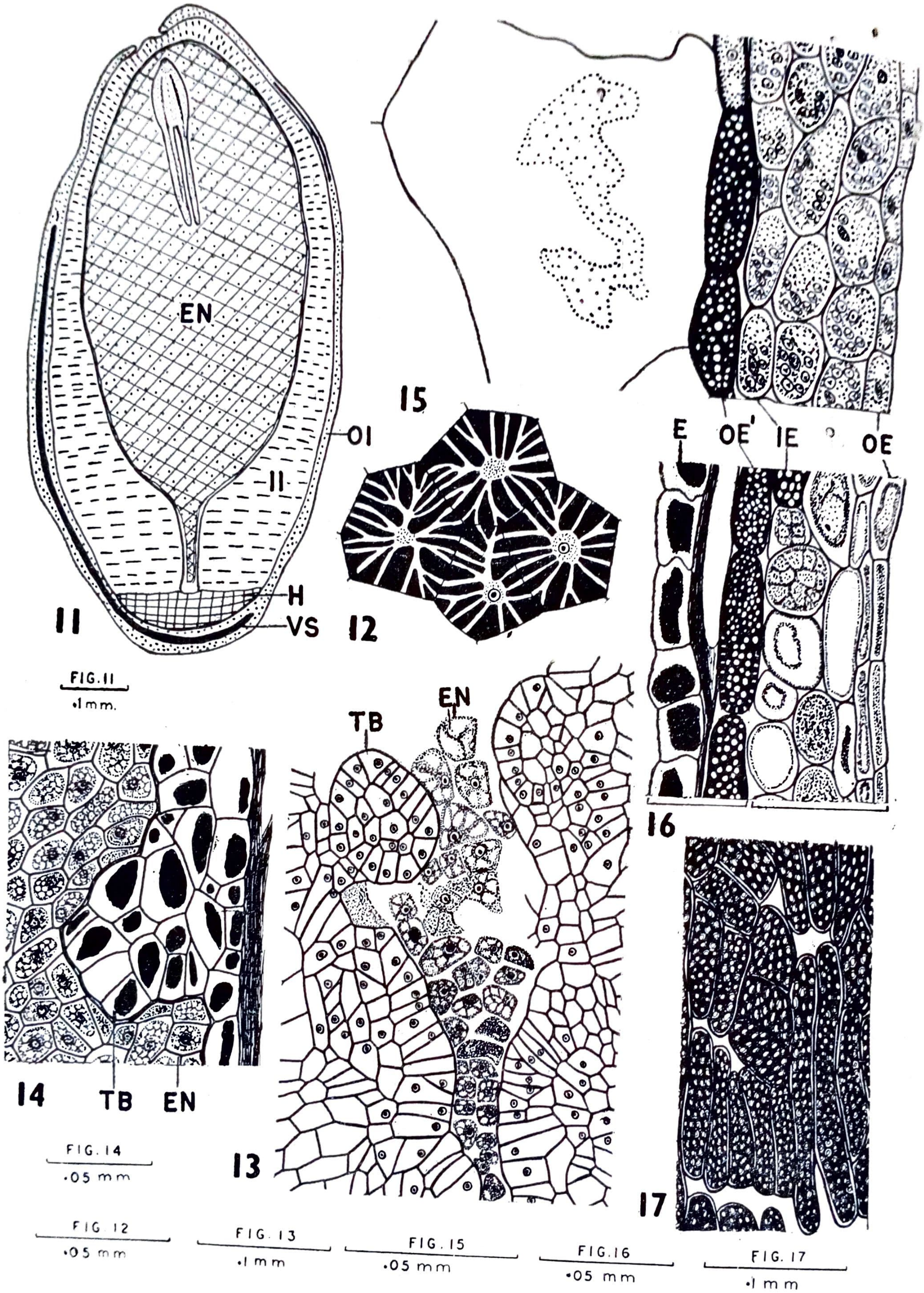
Ovule.—The ovary is generally trilocular and superior but it sometimes shows a tetralocular condition. Each locule has two collateral, pendulous, and nearly anatropous ovules attached to the axile placenta. During ontogeny ovular primordium takes a curve and its apex faces the stigmatic side (Fig. 1). At the megaspore tetrad stage both the integuments surround the nucellus (Fig. 2). The micropyle is formed mainly by the inner integument.

The nucellus, at the time of fertilisation, persists only as a column of parenchymatous cells at the base of the embryo-sac, while its remaining upper portion is consumed by the developing female gametophyte (Fig. 3) as also reported by Thathachar (1953). The inner integument, at this stage, is composed of 7 to 10 layers of cells (Fig. 4) and the cells of its inner epidermis, below the level of the endostome, are tapetum-like. Thathachar (1953) observed an integumentary tapetum in this species, while Banerji and Dutt (1944) did not report this feature. An interesting feature in this species is that the micropyle, except at the top, becomes imperceptible owing to the growth of the inner integument (Figs. 3, 7).

The outer integument is generally composed of 4 to 8 layers of cells (Fig. 4), but in raphe region the number is larger. The cells of this integument contain chloroplasts. Presence of chloroplasts has been recorded also in the outer integument in *Moringa oleifera* (Puri, 1941).

The vascular supply to the ovule reaches the chalaza after descending through the raphe. In the chalazal region it radiates into a number of strands (Fig. 5) which divide further and traverse the outer integument (Figs. 6, 7); they also anastomose to some extent in the integument. No vascular elements are present in the nucellus or the inner integument. Banerji and Dutt (1944) and Thathachar (1953) did not describe this aspect.

The obturator is conspicuous at an early stage of ovule development (Fig. 1). Many of the epidermal cells of the obturator divide transversely (Fig. 8), and form short filamentous structure. The obturator, at the mature embryo-sac stage, is very massive and the cells of its filaments stain deeply than other cells.



FIGS. 11–17. Fig. 11. L.s. young seed. Fig. 12. Cells of hypostase. Fig. 13. L.s. young seed showing only a part of the integumentary tapetum with lobes. Fig. 14. A part of the mature endosperm surrounding one of the integumentary lobes. Fig. 15. L.s. young seed coat showing outer integument and only a portion of inner integument. Fig. 16. L.s. part of mature seed coat. Fig. 17. Sclereids of seed coat developed from outer epidermis of the inner integument in surface view drawn from peelings.

E, tapetum; *EN*, endosperm; *H*, hypostase; *IE*, inner epidermis of outer integument; *II*, Inter integument ; *OE'*, outer epidermis of outer integument; *OE'*, outer epidermis of inner integument; *OI*, outer integument; *TB*, tapetal bulges; *VS*, vascular suppy.

In post-fertilisation stages the obturator stops growth and its deeply stained epidermal cells degenerate. An interesting feature, not mentioned by Thathachar (1953), is the transformation of many cells of the obturator-mass into sclereids with simple pits near the placenta.

Generally a single ovule (though not uncommonly two ovules of different loculi) develops in a mature seed, while the remaining ovules degenerate.

Endosperm and embryo.—Endosperm development is of the Nuclear type. As it develops, the chalazal portion of the embryo-sac penetrates deep in the nucellar column and absorbs it (Figs. 9, 10). The free nuclear endosperm ultimately becomes cellular and fills the entire embryo-sac (Fig. 11). The endosperm occupies a major part of the mature seed and its cells contain reserve products.

The embryogeny could not be studied because of slow development of the embryo and longer period required for fruit development. The developing embryo destroys the cells of the endosperm which come in its way (Fig. 11). The mature embryo is straight and is differentiated into a root-cap, a hypocotyl-root axis, two broad cotyledons, and an epicotyl. The vascular supply is branched in the cotyledons. The cells of the embryo also contain reserve products.

Changes in the chalaza.—The chalaza remains parenchymatous for some time, but approximately at small globular proembryo stage a massive hypostase differentiates below the lower limits of the inner integument and is well marked by the time embryo gets differentiated (Fig. 11). The hypostase is composed of sclereids with simple or ramiform pits and their lumen shows cytoplasmic inclusions (Fig. 12). Rest of the chalaza remains parenchymatous and persists in the mature seed.

Changes in the integuments.—The behaviour of the integumentary tapetum in post-fertilisation is interesting, though Thathachar (1953) did not describe it. The tapetal cells divide in varying planes at a number of places making the endothelium multilayered. The endothelium produces a number of bulges throughout the seed, their number being more in the chalazal region. These bulges in the chalazal region are packed closely and leave only a narrow space for the endosperm (Fig. 13). As the seed develops, these bulges are mostly consumed by the endosperm. In the mature seed, however, some of these bulges may still be seen surrounded by the endosperm (Fig. 14), particularly towards the micropylar end. The endothelium persists as the inner lining of the seed coat (Fig. 16).

The intermediary cells enlarge in later stages, but finally they get crushed. In the mature seed they persist in the form of a membrane.

The cells of the outer epidermis remain thin-walled for a considerable period. They are ultimately transformed into sclereids with simple pits (Figs. 15, 16). The sclereids are variable in shape when seen in surface view. They are elongated tangentially and this elongation may be in plane parallel to the long axis of the seed or somewhat in transverse direction (Fig. 17), the former condition being more prevalent.

The cells of the outer epidermis of the outer integument become flattened after fertilisation (Figs. 15, 16). The mesophyll cells become, more or less, oval in shape and develop intercellular spaces. Some of these cells are transformed into sclereids with simple pits, while others remain thin-walled. The cells of the inner epidermis, excepting the radially elongated cells near the apex, are indistinguishable from the mesophyll cells, and persist in the mature seed.

Mature seed.—Seeds are excarunculate and light-brown in colour. They are 9 to 10 mm long and more or less spindle-shaped. In cases where two seeds develop in a fruit, the seeds are flat on the side facing each other and convex on the other.

The seed coat is thin and is formed of both the integuments. The sclerenchymatous hypostase persists at the chalazal end of the seed. Surrounded by the endothelium layer lie the massive endosperm and a well-developed embryo of the Spatulate type (Martin, 1946).

Summary and Conclusion

The ovules are bitegmic, pendulous and anatropous. Inner epidermal cells of the inner integument in the region of the embryo-sac are tapetum-like. The micropyle is mainly formed by the inner integument. Vascular strands are present in the outer integument.

Development of the endosperm is of the Nuclear type. The hypostase is composed of sclereids. The integumentary tapetum forms a number of bulges at various places. Majority of them are absorbed by the growing endosperm.

The seed coat is formed of both the integuments. The outer epidermal cells of the inner integument form the characteristic sclereid layer of the seed coat. The seeds are excarunculate.

It is concluded that in spite of drupaceous fruit with thick stony endocarp in *Putranjiva roxburghii* the cells of the outer epidermis of

the inner integument are transformed into sclereids, a feature which is characteristic of all the members of the Euphorbiaceae with dehiscent fruits investigated so far. Transformation of the cells of the outer epidermis of the inner integument in sclereids has been reported in *Antidesma menasu* (Singh, 1965) where the fruit is also a drupe.

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