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The ontogeny of the fruit and seed of *Momordica balsamina*

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ABSTRACT

The ontogeny and morphology of the seed and fruit of *Momordica balsamina* L. are discussed in detail and compared with some of the relevant literature. Cucurbitaceous ovules are usually described as anatropous but, according to this study, at least those of some taxa should be regarded as circinotropous.

RÉSUMÉ

L'ONTOGÉNIE DU FRUIT ET DE LA GRAINE DE MOMORDICA BALSAMINA

L'ontogénie et la morphologie de la graine et du fruit de Momordica balsamina *L. sont discutées en détail et comparées avec la littérature qui s'y rapporte. Les ovules des Cucurbitacées sont habituellement décrits comme anatropes mais, d'après cette étude, ceux de certains taxons au moins devraient être considérés comme circinotropes.*

INTRODUCTION

With 17 indigenous genera (Dyer, 1973) the family Cucurbitaceae is well represented in southern Africa. From the vast literature available on this family, it is clear that the fruit and seed morphology as well as seed physiology of these plants is very interesting, especially the behaviour of the so-called 'nucellar membrane' of which there is some disagreement concerning its interpretation. This study was undertaken to gain more knowledge about South African cucurbits and to clarify the placentation, origin of the aril and interpretation of the 'nucellar membrane'.

Momordica balsamina L. was chosen since plants were readily available in the botanical garden of the University of Pretoria. This plant is also of general interest as it has been used by local tribes for medicinal purposes (Watt & Breyer-Brandwijk, 1962). The plant is described by Meeuse (1962) as a perennial herbaceous climber, but in Pretoria the aerial parts are ephemeral and new branches are produced from the subterranean parts every year. Microsporogenesis and pollination were also studied but will be dealt with in another paper.

MATERIAL AND METHODS

The material used for this study was collected from plants growing in containers in the garden of the Department of Botany, University of Pretoria.

For the preparation of the semi-thin sections, female flower buds, pollinated flowers as well as young and mature fruits of different developmental stages, were dissected and fixed in 6% glutaraldehyde in 0,05 mol. dm^{-3} cacodylate buffer. The material was dehydrated and embedded in glycol methacrylate (GMA) according to Feder & O'Brien (1968). The monomer mixture used, was as described by Von Teichman und Logischen & Robbertse (1981). Sections of approximately 2 µm thick, were cut with glass knives on a Reichert OMU 3 ultramicrotome, stained with periodic acid-Schiff's reagent (PAS) (Feder & O'Brien, 1968) using 0,5% 2,4 dinitrophenol hydrazine (DNPH) in 15% acetic acid as a blocking agent. Sections were counterstained for 1–5 minutes in 0,05% toluidine blue in benzoate buffer at pH 4,4 (Sidman *et al.* 1961).

OBSERVATIONS

1. *Ovule development*

The inferior ovary of the female flower is tri-locular with three 'placental ridges' dividing each locule into two pseudo-locules (Fig. 1) In each pseudo-locule there are two pairs of circinotropous ovules. The basal parts of the ovules fuse, thus occluding the locules. Vascular supply to the ovules comes from one central vascular bundle in the distal parts of each of the placental ridges.

Ovule development starts with the initiation of the archesporial cell, which after a periclinal division, forms a parietal cell and a megaspore mother cell. The origin of the inner integument is dermal, whereas the outer integument is of subdermal origin (Fig. 2). The inner integument consists of only two cell layers and forms the micropyle (Figs 5 & 6), whereas the massive outer integument consists of approximately 10 cell layers.

The crassinucellus stems from repeated periclinal divisions of the parietal cell. A nucellar beak is partly formed by periclinal divisions of the nucellus epidermis (Figs 3 & 4). A nucellar canal, which is a very unusual structure, is formed between the megaspore mother cell and micropyle (see discussion).

The megaspore mother cell undergoes considerable elongation during the development of the nucellus (Figs 2 & 3). Since it is the chalazal megaspore that forms the 8-nucleate embryo sac, (Figs 4 & 6) the embryo sac can be regarded as being of the Polygonum type (Maheshwari, 1950).

The synergids are highly vacuolated cells with a very distinct filiform apparatus (Fig. 6), and the egg cell has a dense cytoplasm. The polar nuclei fuse before fertilization (Fig. 6c) and the antipodal cells become appressed in the chalazal end of the embryo

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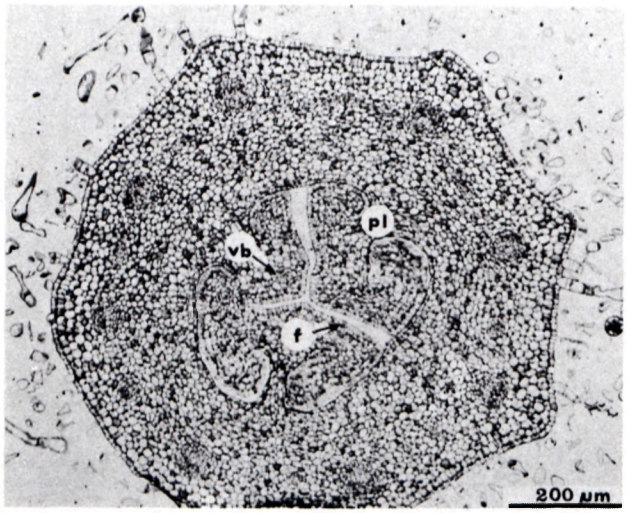

FIG. 1.—Transection of ovary showing three placental ridges. pl, placental ridge; vb, central vascular bundle; f, funicle.

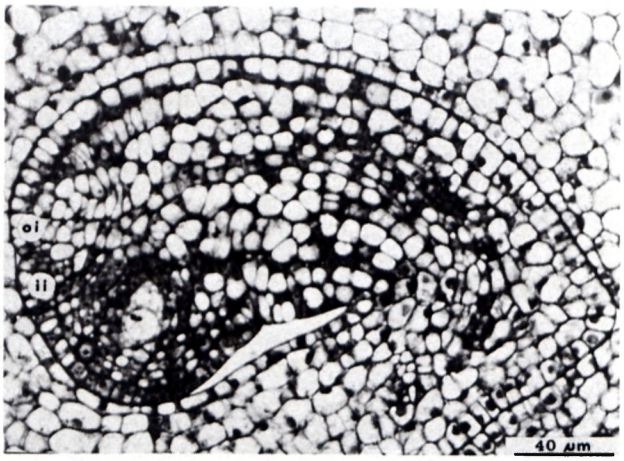

FIG. 2.—Longitudinal section of young ovule showing the megaspore mother cell and origin of the integuments. oi, outer integument; ii, inner integument.

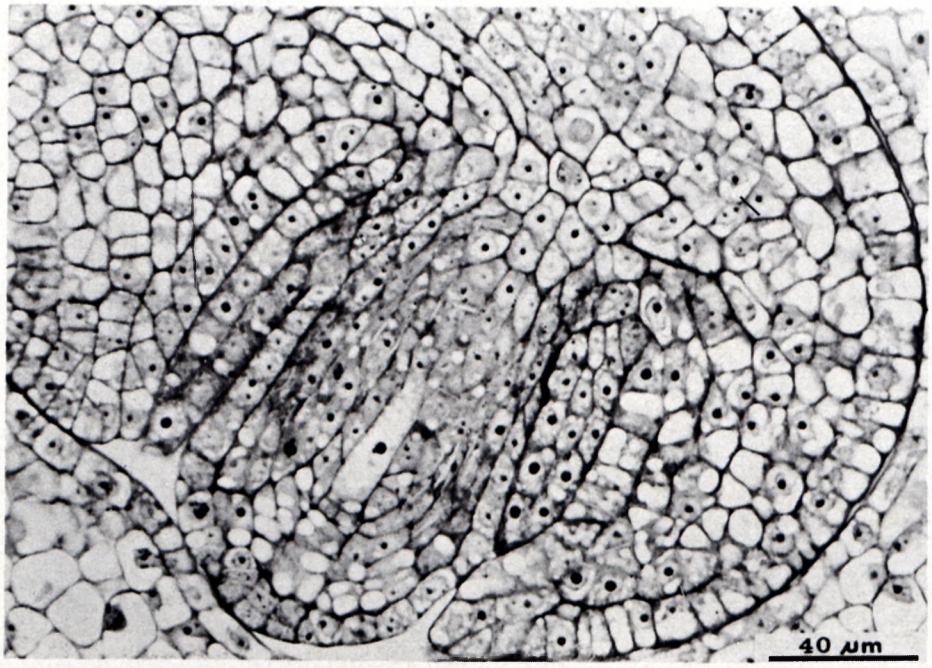

FIG. 3.—Longitudinal section of ovule showing elongated megaspore mother cell.

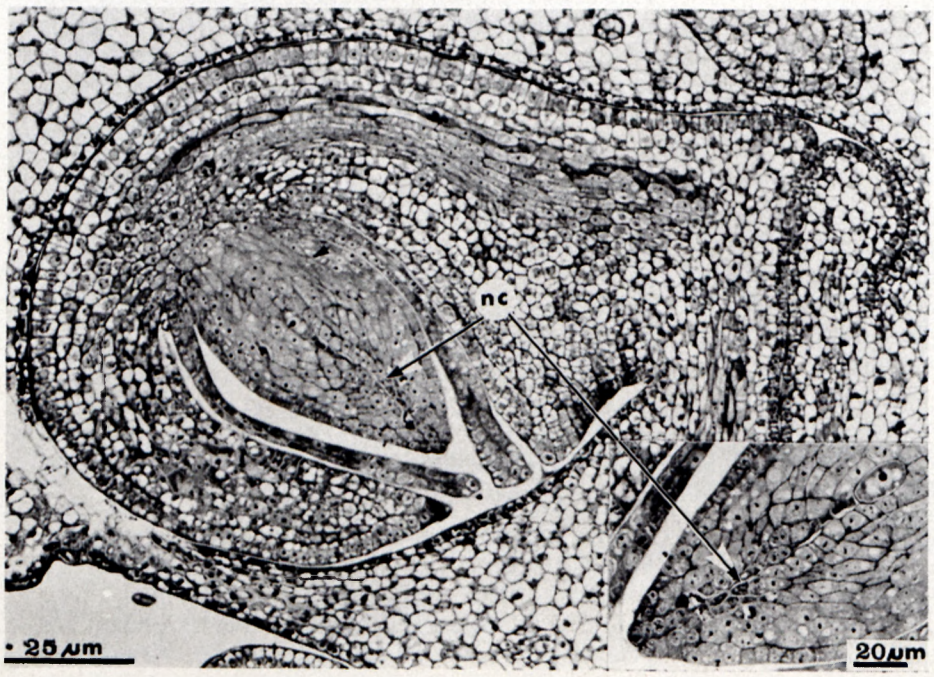

FIG. 4.—Longitudinal section of ovule showing nucellar canal in linear tetrad stage. nc, nucellar canal.

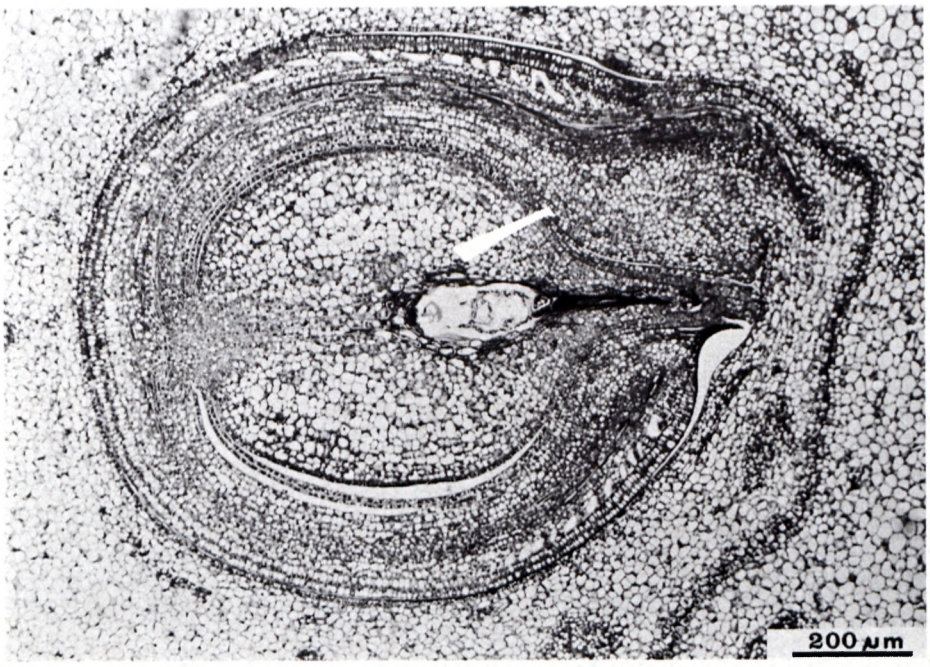

FIG. 5.—Longitudinal section of circinotropous ovule with thick-walled pollen tube in the nucellar beak.

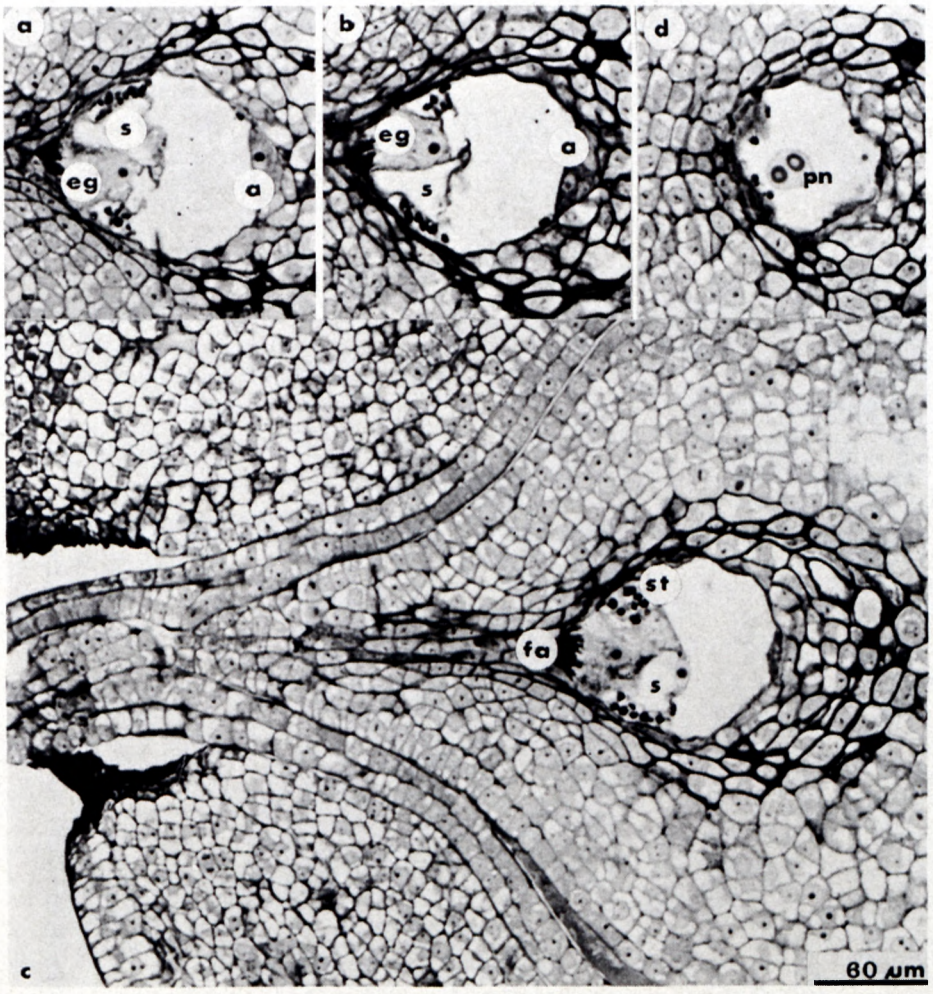

FIG. 6.—Series of sections of ovule showing: a, synergid (s), egg cell (ec) and antipodal (a); b, antipodals (a), synergids (s) and egg cell (ec); c, synergid (s), filiform apparatus (fa) and starch grains in central cells (st) and d, fused polar nuclei (pn).

sac. Starch grains in the central cell seem to be concentrated in the micropylar end (Fig. 6).

As will be described in detail in another paper (in preparation) fertilization is porogamous and the thick-walled pollen tube remains in the nucellar beak (Figs 5 & 7).

2. *Seed Development*

After fertilization, the nucellus continues to develop and fills the fullgrown but immature seed (Fig. 9), but is gradually absorbed by the developing embryo so that only the thick cuticle of the epidermis and appressed cell walls remain in the mature seed (Fig. 9).

The endosperm is of the nuclear type (Fig. 7), but initially it is limited to the micropylar end of the seed where it is confined to the embryo sac wall. No endosperm haustorium was seen. Although most of the endosperm is absorbed by the embryo, at least one layer of well defined cells remains and becomes attached to the outside of the embryo (Figs 8 & 10).

The inner integument remains two cell layers thick, but disintegrates in the maturing seed. The exotesta derives from the outer epidermis of the outer integument by repeated periclinal divisions (Figs 9 & 10). The outermost layer (e-layer according to Kratzer, 1918) consists of columnar cells with thick outer walls and thinner radial and inner walls, staining purple with toluidine blue (Sidman *et al.*, 1961). Below the e-layer are several layers of sclerenchymatous cells, staining greenish-blue with toluidine blue and representing the e''- and e'-layers of Kratzer (1918). Adjacent to the e-layer, cells of the e''-layer are small and globular, with a gradual transition to substellate or stellate cells with very thick walls. The e'-layer is ill-defined and consists of very thick-walled stellate cells.

Three zones can be distinguished in the aerenchymatous endotesta. A zone comprising one to three layers of small, somewhat thick-walled cells is followed by a vascular bundle containing zone of much larger, thin-walled cells. The innermost zone consists of cells with a dense cytoplasm with lipid droplets around the nucleus (Fig. 10).

The embryo is of the onagrad-type (Foster & Gifford, 1974) with a very distinct hypophysis (Fig. 7).

3. *The fruit wall and pseudo-aril*

The fruit develops from an inferior ovary, but for the purpose of this description no distinction will be

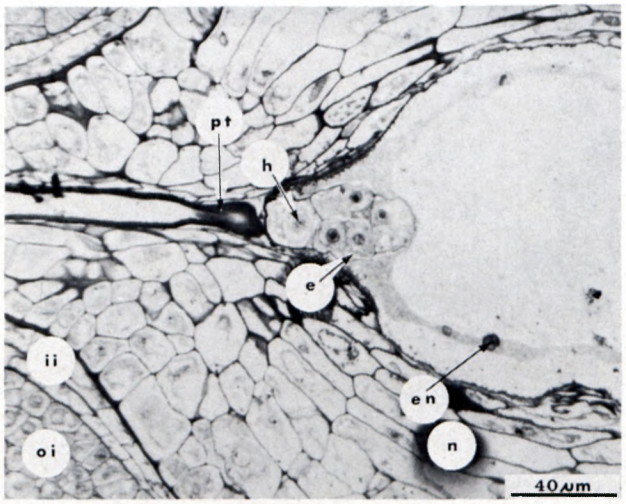

FIG. 7.—Section through a part of young seed showing onagrad-type embryo (e), hypophysis (h) and nuclear-type endosperm (en); pollen tube (pt); nucellus (n); inner integument (ii); outer integument (oi).

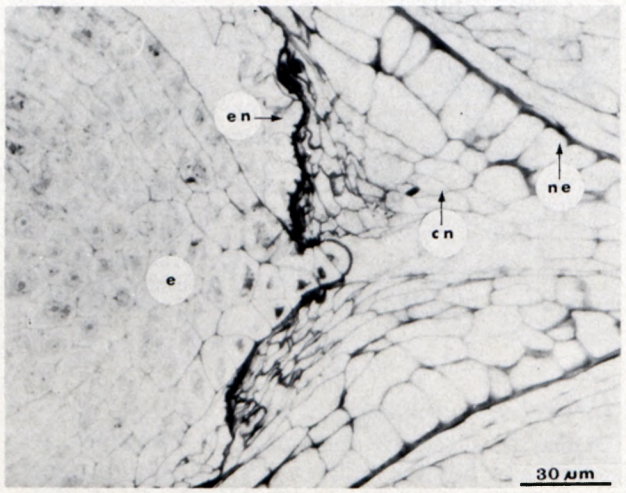

FIG. 8.—Section of part of the full-sized, immature seed showing cutinized epidermal walls of nucellus (ne), crushed nucellar walls (cn) and layers of living endosperm (en), and embryo (e).

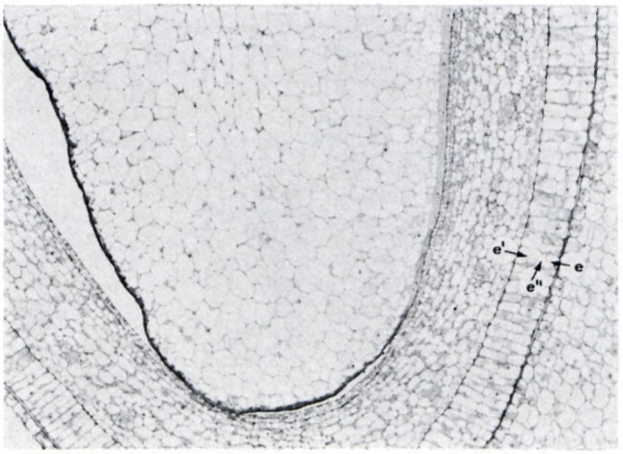

FIG. 9.—Transverse section of a fullgrown but immature seed showing formation of exotesta from outer epidermis of outer integument: e- layer; e'- layer and e''- layer.

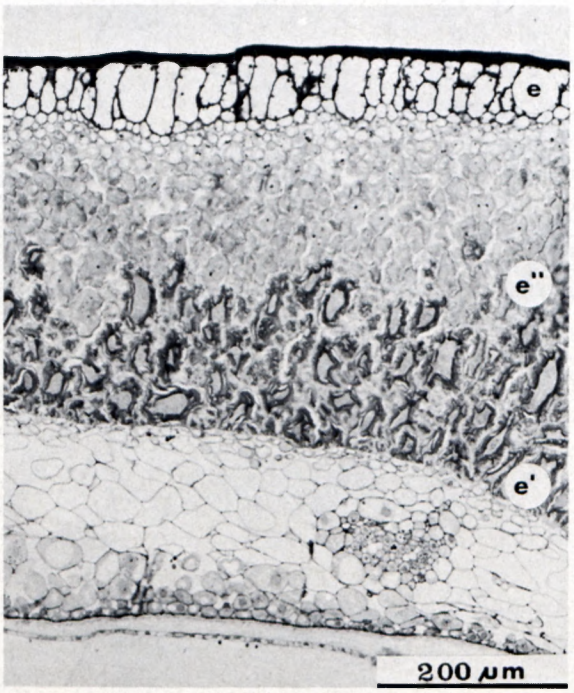

FIG. 10.—Transverse section of testa showing the different layers of the exostesta and endotesta (e- layer; e'- layer and e''- layer).

made between receptacle tissue and carpel tissue since, apart from cell size, it is very difficult to distinguish between these tissues. In the young fruit, six distinct zones are discernible: (1) outer epidermis, (2) hypodermis, (3) parenchymatous zone of large cells, (4) zone of radially elongated cells, (5) vascular bundle containing zone and (6) inner epidermis. The vascular tissue is found in the fifth layer, to the inside of the radially elongated cells (Fig. 11). At this stage placental tissue has merged with the funicles and endocarp, leaving each seed in a small cavity enveloped by an epidermis (referred to as zone 6 above).

In the maturing fruit, periclinal divisions of cells in the third zone causes its extension and shortening of the zones towards the inside (Fig. 12).

Autolysis causes the destruction of most of the cells in the fifth zone. Lysis starts from the inside of the fruit (Fig. 13), but the inner epidermis around the seeds as well as a number of adjacent starch containing cells (Fig. 15) are left intact and forms the pseudoaril. During the destruction of the fifth zone, the vascular tissue remains as loose strands in the mulch (Fig. 14).

In the ripe fruit, starch grains in the pseudoaril are hydrolyzed to sugars. Red chromoplasts are formed (see also Roderiquez *et al.*, 1976) resulting in a dark red, sweet tasting pseudo-aril around each seed (Figs 16 & 17). Ripe seeds are displayed after rupture of the fruits and recurving of the wall fragments.

DISCUSSION

According to Corner (1976), the ovules of the Cucurbitaceae are anatropous. We, however, interpret the ovules of *Momordica balsamina* as being circinotropous (Fahn, 1967). The reason for this difference in interpretation lies in differing interpretations of the anatomy of the fruit. Esau (1977) presents a diagram to explain her interpretation of the fruit of *Citrullus vulgaris*. In the diagram it is clearly shown that the three vascular bundles in the placentae derive from the fusion of the ventral bundles of adjacent carpels, but in the text she states: 'The margins of the carpels are incurved first centripetally and thus form the partitions between the locules. A second curvature carries the

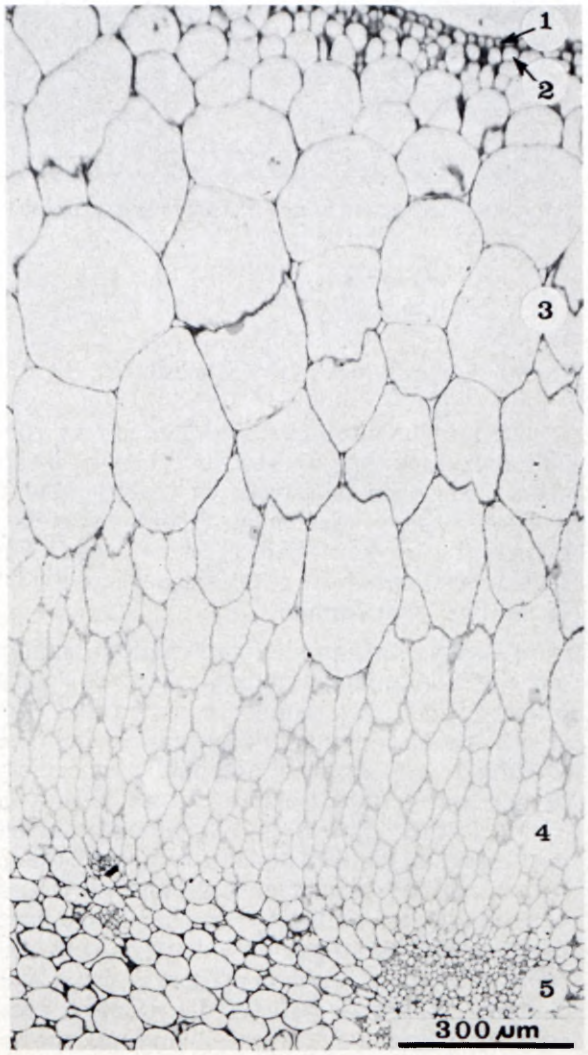

FIG. 11.—Transverse section of young fruit wall showing different zones (1–5).

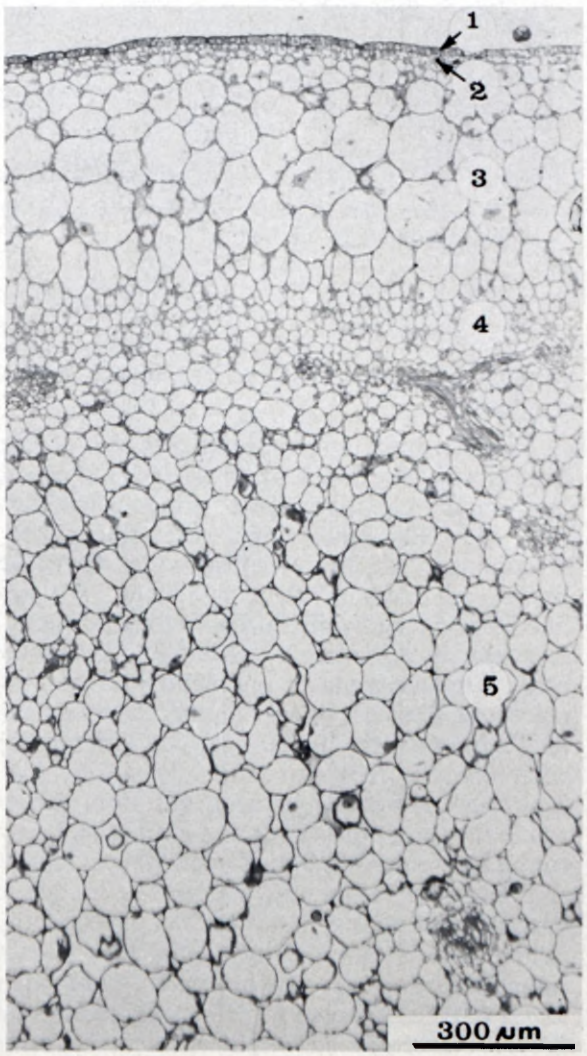

FIG. 12.—Transection of maturing fruit, showing different zones (1–5).

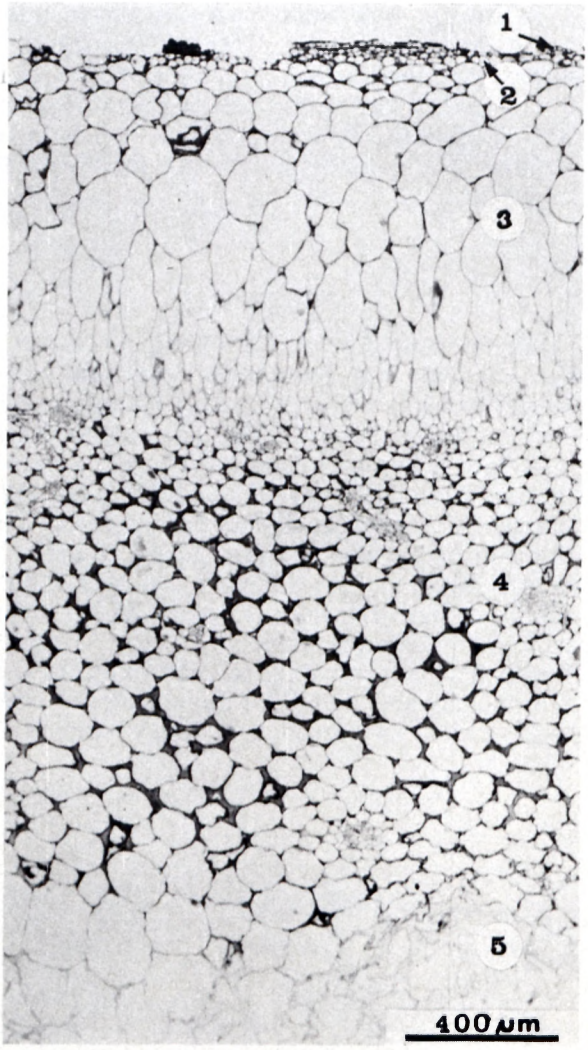

FIG. 13.—Transection of almost ripe fruit where lysis starts from the inside of the fruit.

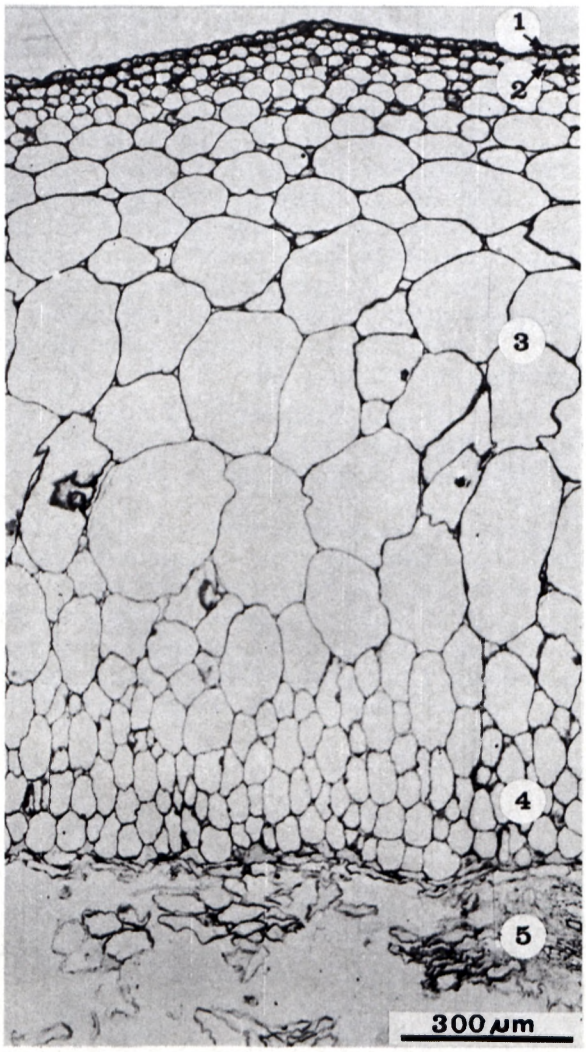

FIG. 14.—Transection of ripe fruit showing disintegrated 5th zone.

carpellary margins centrifugally so that each *locule* is divided'.

This interpretation conforms with that of Rendle and Judson as quoted by Puri (1954). Puri's own interpretation is that the placentation is parietal and that the 'Placental ridges are interpreted as "compound" structures, consisting partly of septal tissue and partly of placental tissue'. We cannot indulge in a lengthy discussion on the merits of these different interpretations. Whether the placental ridges are true septa or outgrowths of the parietal placentae, does not appear to affect our view that in *M. balsamina* the ovules can be interpreted as circinotropous. The reason for this view is that the centrifugal structures growing out from the point where the three central bundles are situated, are not continuous along the longitudinal axis of the ovary and must, therefore, be interpreted as the basal parts of the funicles (Figs 4 & 5). Consequently the funicle almost encircles the ovule, thus forming a circinotropous ovule. Circinotropous ovules are also found in the Cactaceae and Plumbaginaceae (Fahn, 1967). This might be of phylogenetic importance, since, like the Cucurbitaceae, the Plumbaginaceae is one of the few exceptions where bitegmic seeds are found in the Sympetalae (Maheshwari, 1950).

The nucellar canal observed here is very unusual although, according to Kirkwood (1904), a similar canal has been observed by Amici (1842) in *Cucurbita pepo*. Schleiden (1944) rejected this observation by Amici but, as can be seen in Fig. 4, such a canal does exist at the stage where the linear tetrad has been formed.

In recent work on seed germination, mention is made of a 'nucellar membrane'. Mayer & Poljakoff-Mayber (1975), for instance, mention that 'Brown (1940) has shown that the nucellar membrane of *Cucurbita pepo* shows differential permeability to oxygen and carbon dioxide'. Brown (1940), however, refers to Höhnel's (1876) anatomical studies on membranes of the seed of *Cucurbita* where the latter author describes the 'inner membrane' as consisting of several layers, including remnants of the nucellar tissue as well as a layer of endosperm cells. In the mature seed of *M. balsamina* there is also a layer of cutinized epidermal walls of the nucellus, crushed nucellar walls and one layer of living endosperm left between the embryo and seed coat (Fig. 8) which conforms to Brown's (1940) concept of a 'nucellar

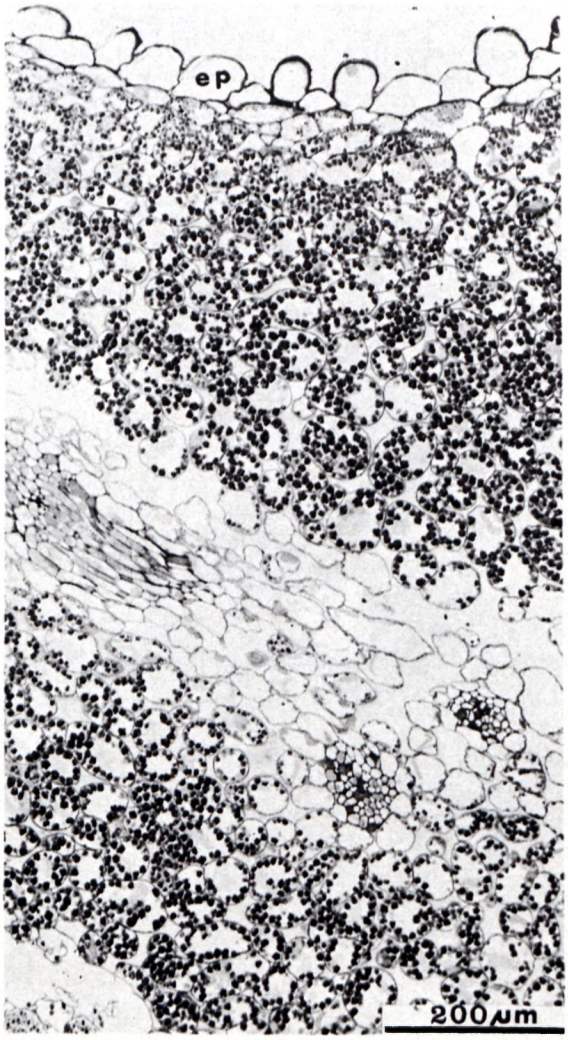

FIG. 15.—Section of maturing (light orange) pseudo-aril with starch containing cells. ep, epidermis facing seed.

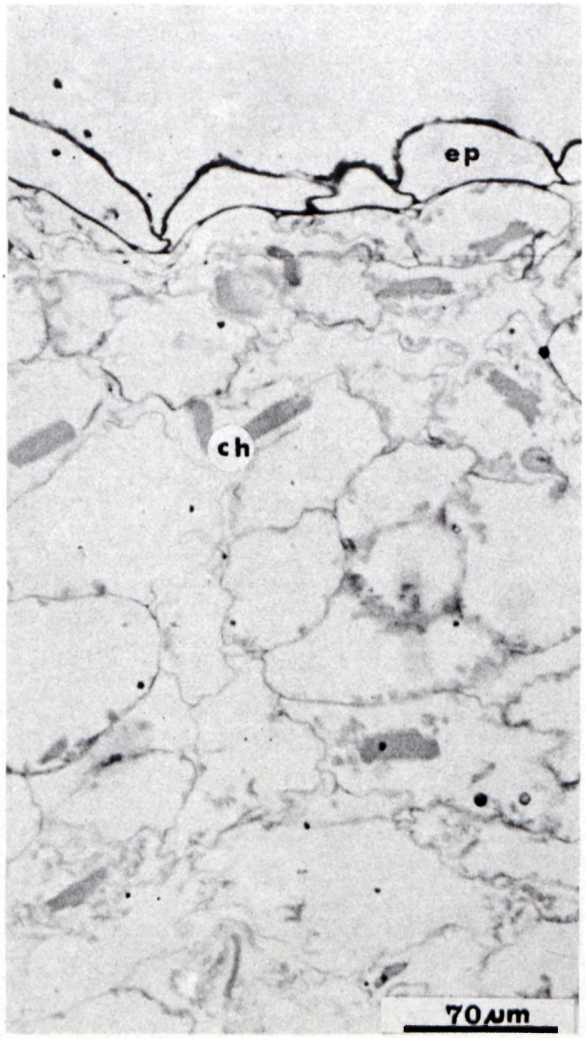

FIG. 16.—Section of mature (dark red) pseudo-aril where the starch grains have been hydrolised. ch, chromoplasts; ep, epidermis facing seed.

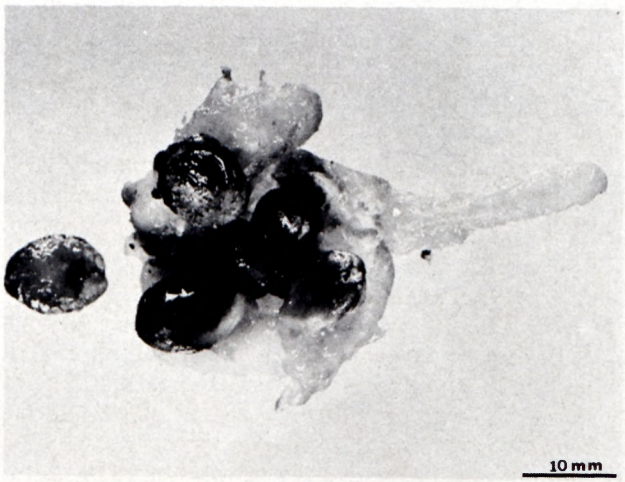

FIG. 17.—Ruptured fruit displaying ripe seeds each in a dark red pseudo-aril.

membrane'. This latter term should not be used since the remnants of the nucellus consist of appressed cell walls and the only living cells that could have any physiological influence on the seed are endosperm cells.

The outer wall layers in the ovary of *M. balsamina* conform to a great extent with those described by Kraus (1949) for *Bryonia dioica*. According to Corner (1976), the pseudoaril in *Momordica* derives from placental tissue but, as can be seen from Figs 2 & 5, the tissue adjacent to the seed on the antiraphe side is placental plus funicular, and that on the chalazal and rafe sides, of carpellary origin.

The broad, persistent pollen tube is mentioned by different authors e.g. Maheshwari, 1950 and Davis, 1966. Longo, according to Maheshwari (1950) and Kratzer (1918) believes that the pollen tube could have a haustorial function which seems possible if its contact with the suspensor in Fig. 7 is considered; however, more work is needed before an opinion can be formed.

The onagrad-type embryo of *M. balsamina* conforms with those of other Cucurbitaceae as described by Kirkwood (1904) and Davis (1966). No endosperm haustorium was seen (Chopra, 1955).

CONCLUSION

Seed morphology of *M. balsamina* differs very little from that of other investigated representatives of the Cucurbitaceae. As far as the placentation is concerned, we partly agree with the interpretation by Puri (1954). We differ from him, however, in regarding the centrifugal outgrowths, on which the ovules are borne, as part of the funicle with the implication that the ovules are interpreted as circinotropous.

Our interpretation of the 'false aril' (Corner, 1976) is that it is of heterogeneous origin, consisting of funicular, placental and carpellary tissue.

The term 'nucellar membrane' (Mayer & Poljakoff-Mayber, 1975) is misleading, since this membrane consists of a cutinized epidermal wall of nucellus, appressed cell walls of nucellar tissue and a layer of live endosperm cells adjacent to the embryo.

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