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DEVELOPMENT OF OVULE AND SEED IN BEGONIA SQUAMULOSA HOOK. F.

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SUMMARY

In spite of the dizonate structure of the ovule primordium, the ovule is crassinucellate. Both integuments remain two-layered and become crushed during seed development, the outer layer which differentiates into the exotesta excepted. A transverse ring of specialised testa cells helps to form a special zone of rupture by which the operculum lifts off.

1. INTRODUCTION

The Begoniaceae form a natural family largely consisting of sappy herbs and suffrutices. The family occurs in the tropical and subtropical zones and according to the most recent classifications comprises the 3 genera *Begonia, Symbegonia* and *Hillebrandia.* The many-seeded fruit is mostly a capsule, occasionally a berry.

The structure of begoniaceous ovules and seeds was already studied by MÜLLER (1847) and WARMING (1878). The most important work is of more recent date: SAND (1921), MAURITZON (1936), MEREMINSKY (1936), SWAMY & PARAMESWARAN (1960), SEITNER (1972), and MAHESWARI DEVI & CHANDRASEKHAR NAIDU (1979). The accumulated data were summarized by NETOLITZKY (1926), DAVIS (1966) and CORNER (1976). The ovules are bitegmic, anatropous and crassinucellate. The chalazal megaspore develops into the embryo sac. During the development of the seed coat all layers, the exotesta excepted, are crushed and obliterate.

The endosperm is initially nuclear to become cellular later and is subsequently resorbed with the exception of one layer containing aleuron grains. The embryo is relatively large. Externally the seeds of *Begonia* show three parts, *viz.* the operculum, which comprises the micropyle and hilum, the collar cells and the remainder of the seed. The operculum functions as a seed-lid, which is lifted during germination thus clearing the way for the emerging seedling (BOUMAN & DE LANGE 1982, 1983).

This investigation was carried out, because within the scope of an extensive, micromorphological inquiry into the characteristics of the testa in Begoniaceae additional information about its development and internal structure was required.

2. MATERIALS AND METHODS

Ovaries in various stages of development and mature seeds of *Begonia squamulosa* s.l. were provided by the Department of Plant Taxonomy, Agricultural University, Wageningen. This material was sampled at various times after self-pollination of plants, collected in Gabon by F. J. Breteler and J. J. F. E. de Wilde in 1978. These plants are cultivated at the Department of Horticulture of the same University and there registered as accessions T 1194B, T 1207D and T 1210B.

Similarly seeds from *B. meijeri-johannis* Engler were obtained from a plant collected by J. J. F. E. de Wilde in Kenya in 1979. It is cultivated as T 1260.

Craf or Allen-Bouin mixtures were used as fixatives. Phloroglucinol-HCl, Sudan IV and JKJ were applied as specific stains. SEM observations were made with a Cambridge Stereoscan mark II[a]. The dry or fixed material was scanned after about 3 minutes of sputter-coating with gold-palladium. The fresh material was scanned without previous treatment.

3. RESULTS

3.1. Ovule ontogenesis

The ovule primordia arise subdermally from the placenta and exhibit a dizonate structure (*fig. 1A*). In such a build-up the ovule is initiated by periclinal divisions in the second tunica layer. The primordium consists mainly of tissue derived from the second layer (L2), which is surrounded by the anticlinally dividing cells of the dermal layer (*fig. 1B, C*). Already in an early stage of development the primordium begins to show the anatropous curvation.

The subdermal archespore cell divides into a parietal cell and a megaspore mother cell. The parietal tissue remains small and consists of only a few cells. The megaspore tetrad is linear and the chalazal cell develops into the embryo sac (e.s.). The nucellus is rather short at first but soon it becomes oblong as a result of mitotic activity of cells below the e.s. These cells arrange themselves in more or less parallel rows, and later stretch longitudinally. They are supposed to have a transport function. The anatropous curvation is the result of cell divisions at the dorsal side and a concomitant enlargement of cells of the outer layer. When the curvation is about 180° both integuments are initiated simultaneously (*fig. 1D*). The inner integument (i.i.) develops first at the abaxial side from 1 or 2 cells which later form a row which extends to develop into a complete ring wall (*fig. 1E, F, G*). From the beginning the i.i. is two cells thick and remains so except in its micropylar part where it becomes multilayered when the ovule approaches maturity (*fig. 2A, B*). The outer integument (o.i.) also develops from one or two epidermal cells and is also two layers thick except at its tip (*fig. 1E, F, G*). Soon it overtakes the i.i. (*fig. 1F*) to form the exostome. The cells of the inner layer become somewhat flattened, while those of the outer layer enlarge substantially (*fig. 1G, 2B*). The micropylar part becomes multilayered mainly by divisions of the cells of the inner layer (*fig. 2B*). During the develop-

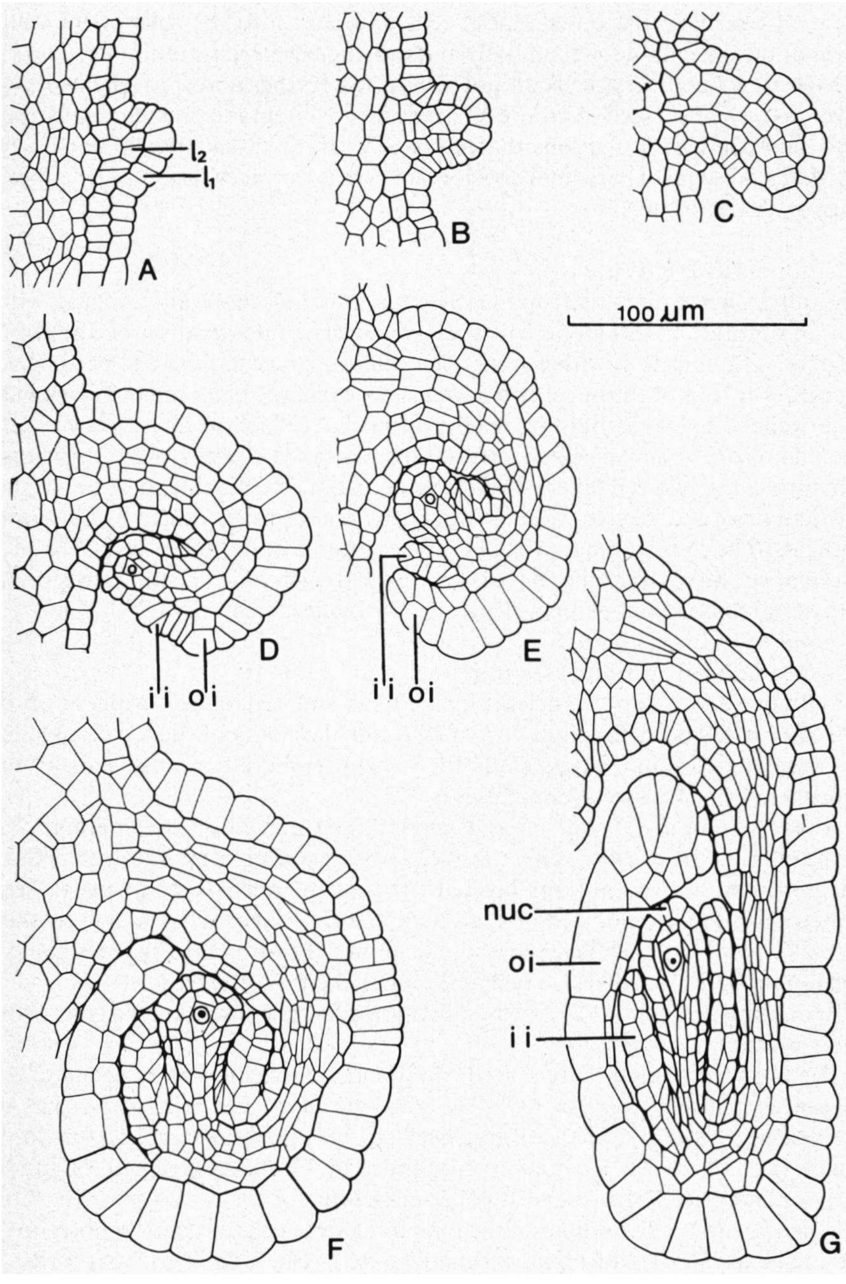

Fig. 1. *Begonia squamulosa*, l.s. of developing ovules.
In all figures L1 and L2: dermal and subdermal layer, emb = embryo, end = endosperm, ex = exotesta, nuc = nucellus, i.i. = inner integument, o.i. = outer integument, r = raphe.

ment of the ovule the outer raphal cells and later also the cells of the outer layer of the o.i. enlarge substantially and rather early become tanniniferous (*fig. 1D-G*). In general these cells do not divide any further and form the exotestal layer in the mature seed. At the level of the future hilum the funicular epidermal cells show periclinal divisions to keep pace with the radial stretching (*fig. 1F, G*). After subsequent periclinal divisions a tissue is formed which has the appearance of an aril primordium.

3.2. The mature ovule

The full-grown ovule is anatropous, bitegmic, crassinucellate and elongate, without an obturator. Actually the ovule has undergone a curvation of about 270 degrees. The funicle is rather long. The cells surrounding the e.s. become resorbed. The cells of the nucellar epidermis have enlarged and remind somewhat of an endothelial layer, but the pseudoendothelial cells show large vacuoles and become resorbed shortly after fertilisation has taken place. Both integuments remain mainly two cell layers thick. The o.i. is weakly developed at the raphal side and protrudes beyond the i.i. The zig-zag micropyle is formed by both integuments. The endostome is orbicular, the exostome of irregular form. The provascular strand of the raphe consists of stretched cells, which do not differentiate into mature xylem and phloem elements. The ovule is free of starch.

3.3. Seed development and mature seed

Initially the endosperm is nuclear (*fig. 2C*) and situated at the periphery of the e.s. After it has become cellular (*fig. 2D*) it fills the cavity of the e.s. completely by repeated divisions. Subsequently the growing embryo consumes most of the endosperm till only one layer remains (*fig. 2E*).

Already at an early stage of seed development the enlarged epidermal cells of the nucellus are crushed, only the nucellar cuticle remaining discernible. Both integuments are two- to three layered. At first the inner layer of the i.i. and somewhat later the outer layer of the i.i. and the inner layer of the o.i. are crushed (*fig. 2C, D*). The outer layer of the o.i. ultimately turns into the mechanical layer of the seed. Its cells have thickened inner and radial walls.

According to SAND (1921) the lignification proceeds simultaneously with the endosperm development.

The brown-coloured mature seed is oblong and its coat has a reticulate appearance. It measures about 0,8–1,1 × 0,4–0,5 mm. The micropylar part of the seed is covered by a small aril-like swelling, which develops around the whole funicle (*fig. 3A, B*). The operculum is bounded by a transverse ring of longitudinally oriented cells, the so-called collar cells, which have an average length of 170 μm (*fig. 3B*). The outlines of the remaining testa cells are mostly polygonal; they have a diameter of about 80 μm (*fig. 3C*). The outer periclinal walls of the exotesta remain unthickened and are covered with an inconspicuous cuticle. These walls have become more or less squashed against the thickened radial and inner periclinal walls and rupture easily (*fig. 3C, D, F*). The lignified thickenings are layered and contain many branched pits (fig. *2E, 3E, 4A, B, C*). In

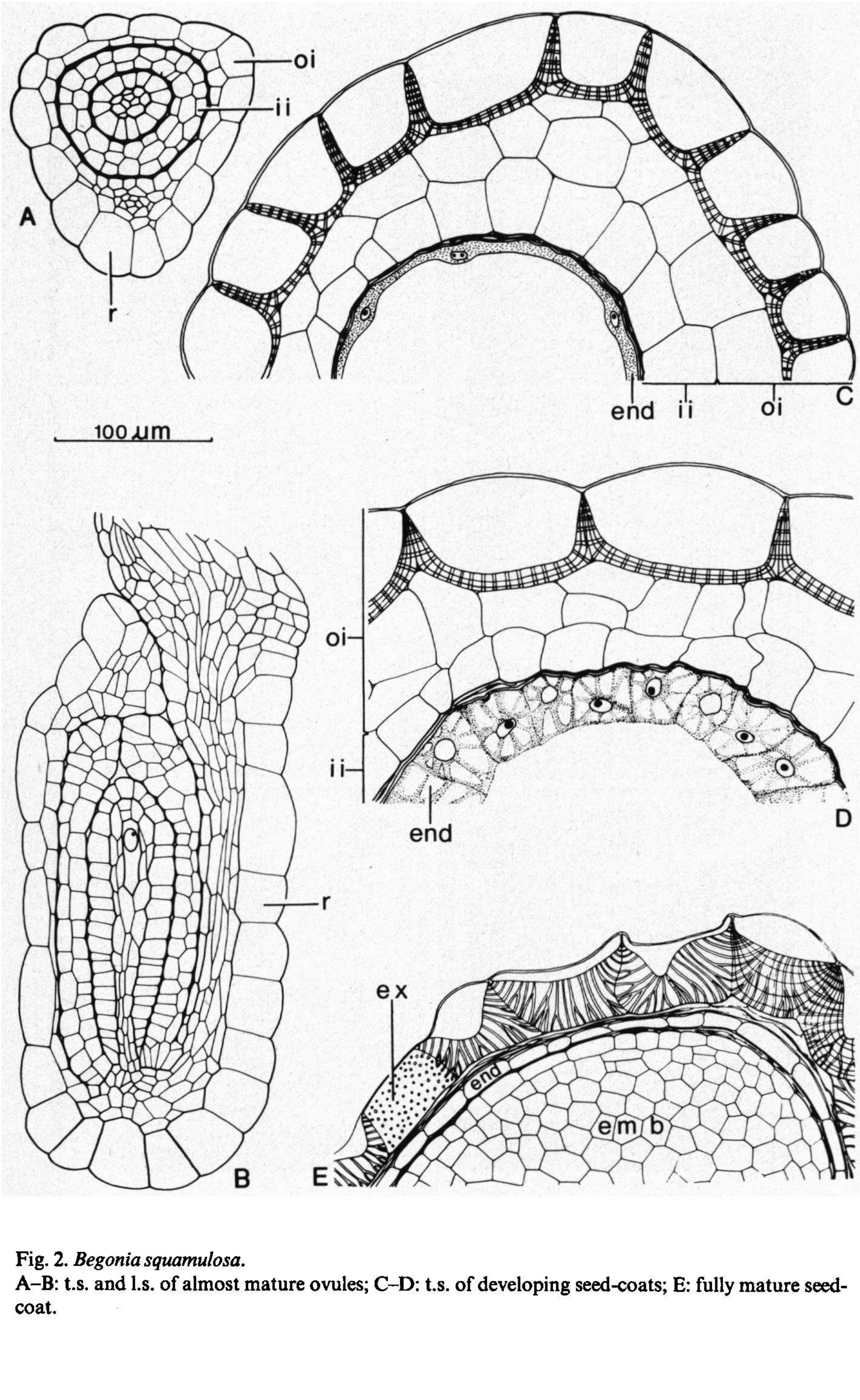

Fig. 2. *Begonia squamulosa.*
A–B: t.s. and l.s. of almost mature ovules; C–D: t.s. of developing seed-coats; E: fully mature seed-coat.

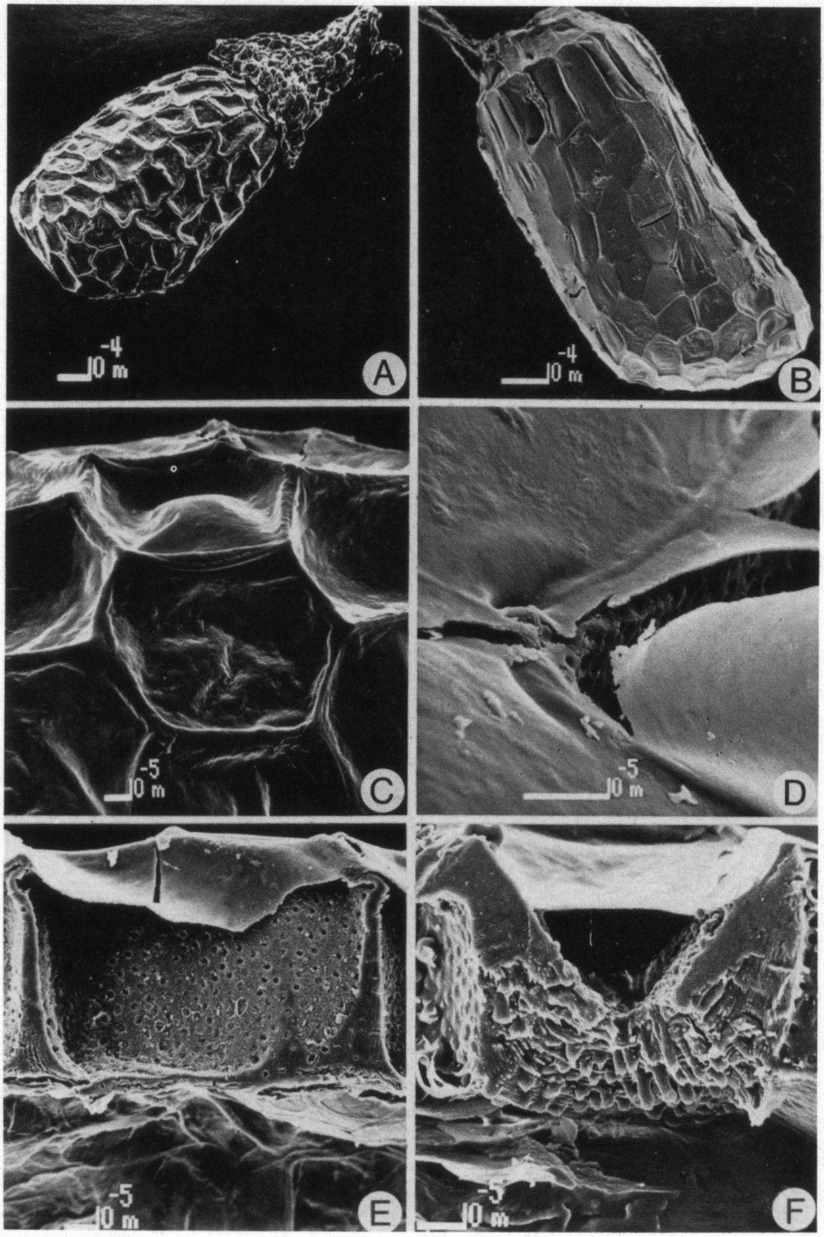

Fig. 3. Seeds of *Begonia squamulosa*, SEM photomicrographs. A: mature seed with aril-like tissue covering the operculum; B: mature seed with aril removed, showing operculum; C: detail of seed-coat in surface view; D: torn seed-coat; E: cross section of developing seed-coat; F: cross section of mature seed-coat.

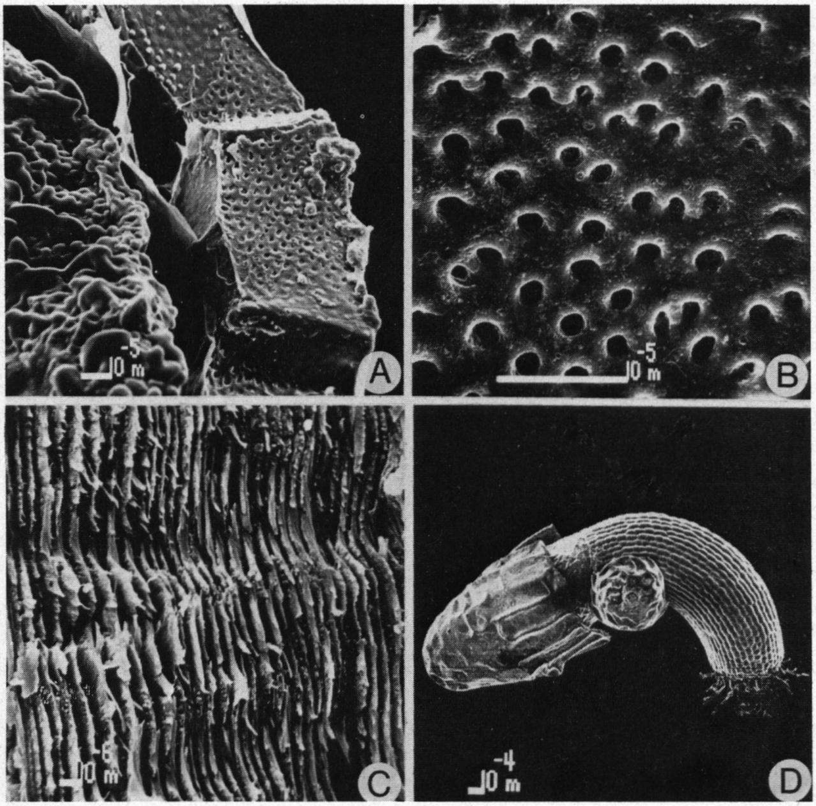

Fig. 4. SEM photomicrographs of parts of seeds.
A, B, C: *Begonia squamulosa*, t.s. of mature seed-coat, detail of pits and detail of wall striation, respectively; D: *Begonia meyeri-johannis*, germinating seed showing root and operculum.

the exostomal region the mechanical tissue is multilayered, possibly to reinforce the operculum. At the inside of the exotesta vestiges of the crushed layers of the integuments and nucellus can be observed.

The endosperm contains oil and aleurone grains according to SAND (1921) and SWAMY & PARAMESWARAN (1960). Between endosperm and seed-coat the nucellar cuticle still persists. The straight embryo also contains oil and aleurone and fills the interior of the seed completely.

When *Begonia* seeds germinate the operculum is lifted after the splitting of the middle lamellae of the cell walls between operculum and collar cells as is shown in *fig. 4D*, showing a germinating seed of *Begonia meyeri-johannis*.

DISCUSSION

The ovule of *Begonia squamulosa* is of rather simple construction. WARMING (1878) already discovered the dizonate structure of its primordium in *B. heracleifolia* and the simple build-up of the ovule.

Also in the genus *Hillebrandia* (MAURITZON 1936) simple ovules occur, which closely correspond with those of *Begonia*. The ovules of both genera have a somewhat elongated nucellus, are crassinucellate and have two-layered integuments. The small seeds of the genus *Symbegonia* suggest that its ovules are also of simple construction or may even be more reduced.

The combination of a dizonate structure and parietal cells is rare. It has been recorded in Marantaceae (GROOTJEN 1983) and *Myosurus minimus* (Ranunculaceae) (BOUMAN, unpublished). In the latter case the transition of a trizonate to a dizonate structured primordium must have taken place within the family.

From the above one can conclude that the ovules of Begoniaceae are rather simplified and derived. After fertilisation has taken place most or all cells of the ovule do not divide any longer and seed development is mainly brought about by cell enlargement and differentiation. The ovules do not show a great deal of differentiation and the seeds are of rather simple build. This is expressed in the presence of a seed coat which is mainly crushed and obliterated, the exotesta and the thin layer of endosperm excepted. A further reduction is found in *B. froebeli* (SOLTWEDEL 1882) and *Hillebrandia* (PRITZEL 1898) where the endosperm is absent in the mature seed. Likewise the vascular bundle is poorly developed and does not contain differentiated elements. The operculum is a seed character of a more complex nature and represents an adaptation to germination.

In most recent systems the Begoniaceae are placed within the parietalean alliance which is often called Violales. However, the most characteristic parietalean families have rather large and complex seeds provided with an exotegmen. As in the Begoniaceae the Datiscaceae have ovules with two-layered integuments and seeds with an operculum. Another parietalean taxon with small seeds and two-layered integuments is the family of the Droseraceae. Here the dermal cells of the nucellus enlarge to assume an endothelial character. In the Droseraceae the simplified seed has a variable seed-coat structure. The genera *Drosera* and *Dionaea* have opercula, but these are of endostomal origin. From the viewpoint of their seed anatomy, the Datiscaceae are clearly the closest relatives of the Begoniaceae.

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REFERENCES

BOUMAN, F. & A. DE LANGE (1982): Micromorphology of the seed coats in Begonia section Squamibegonia Warb. *Acta Bot. Neerl.* **31**: 297–305.
— & — (1983): Structure and micromorphology of Begonia seeds. *The Begonian* **50**: 70–78.
CORNER, E. J. H. (1976): *The seeds of Dicotyledons.* 2 vols. Cambridge.
DAVIS, G. L. (1966): *Systematic embryology of the angiosperms.* New York, London, Sydney.

GROOTJEN, C. J. (1983): Development of ovule and seed in Marantaceae. *Acta Bot. Neerl.* **32**: 69–86.

MAKESWARI DEVI, H. & K. CHANDRASEKHAR NAIDU (1979): Embryological studies in the family Begoniaceae. *Ind. J. Bot.* **2**: 1–7.

MAURITZON, J. (1936): Zur Embryologie einiger Parietales-Familien. *Svensk Bot. Tidskr.* **30**: 79–113.

MEREMINSKY, H. (1936): Über Embryosackentwicklung bei Begonia incana Lindl. (Ein Beitrag zur Embryologie der Gattung Begonia). *Bull. Intern. Acad. Polon.* **B1**: 53–92.

MÜLLER, K. (1847): Beiträge zur Entwicklungsgeschichte des Pflanzenembryos. *Bot. Zeit.* **5**: 753–761.

NETOLITZKY, F. (1926): Anatomie der Angiospermen-Samen. In: K. Linsbauer (ed.) *Handbuch der Pflanzenanatomie* Band X, **4**. Berlin.

PRITZEL, E. (1898): Der Systematische Wert der Samenanatomie, insbesondere des Endosperms bei den Parietales. *Bot. Jahrb.* **24**: 348–394.

SAND, W. (1921): Beiträge zur Kenntnis der Begoniaceen. *Flora* **114**: 329–384.

SEITNER, P. G. (1972): Some observations on Begonia seeds. *The Begonian* **39**: 47–55.

SOLTWEDEL, F. (1882). Freie Zellbildung im Embryosack der Angiospermen mit besonderer Berücksichtigung der hierbei stattfindenden Vorgänge der Kernteilung. *Jen. Zeit. Nat.* **15**: 341–380.

SWAMY, B. G. L. & N. PARAMESWARAN (1960): A contribution to the embryology of Begonia crenata. *J. Indian bot. Soc.* **39**: 140–148.

WARMING, E. (1878): De l'ovule. *Ann. Sci. nat. Bot.* **5**: 177–266.