POST-FERTILIZATION DEVELOPMENT OF THE OVULE IN *BARLERIA CRISTATA* LINN.

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INTRODUCTION

THE literature on the embryology of the Acanthaceae has been reviewed by Johri and Singh (1959). The earlier investigations of Hartmann (1923) and Schürhoff (1926) on the genus *Barleria* are very meagre. Phatak and Ambegaokar (1955, 1956) have described the development of the female gametophyte, endosperm and embryo in *B. prionitis*. They report the following special features in their work: (1) the antipodal cells which are usually ephemeral in the Acanthaceae not only persist in this plant but also divide to produce 8–13 cells; (2) even the first division of the central endosperm chamber is followed by wall formation; (3) the micropylar endosperm haustorium is invaded by the cellular endosperm proper; and (4) supernumerary embryos are formed by the budding of the suspensor.

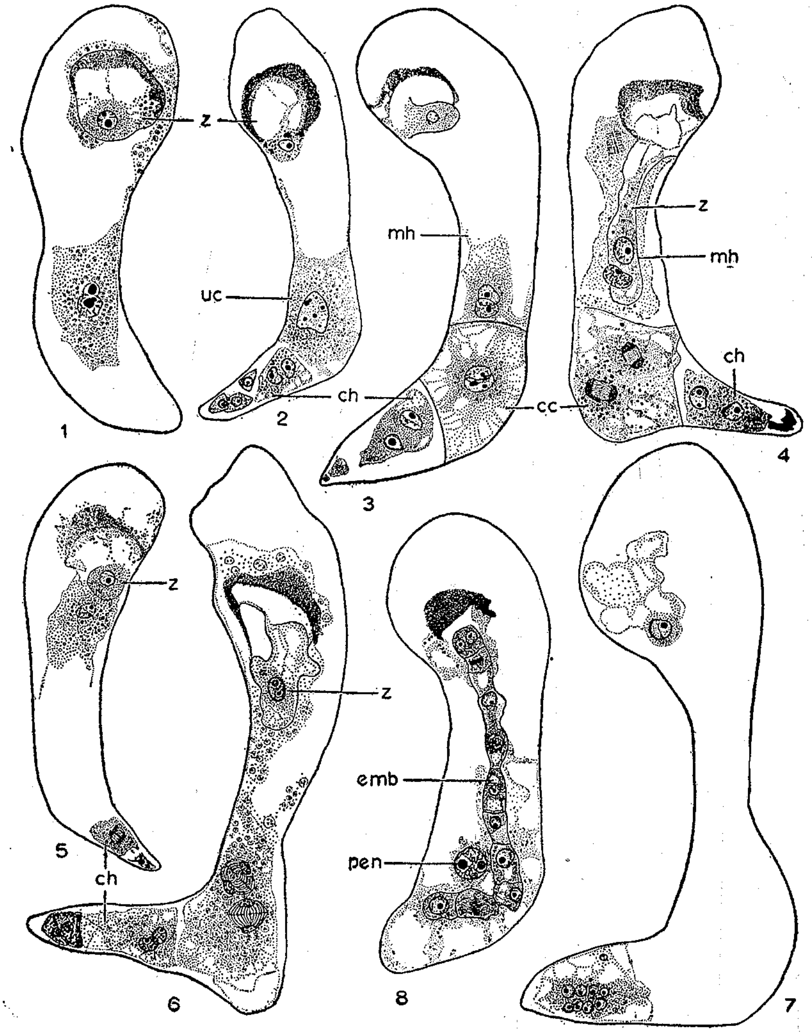
MATERIAL AND METHODS

The material was collected periodically from plants growing in the University campus, Delhi, during September to February, 1954–57. The ovules were dissected out of the ovaries and fixed in formalin-acetic-alcohol at various stages of development after fertilization. The usual methods of dehydrating and embedding were followed and sections were cut at a thickness of 10–15 μ. Heidenhain's haematoxylin was the most common stain used followed by a counterstain with erythrosin, fast green or orange G. The endosperms were dissected out under a stereoscopic microscope and mounted in a medium composed of acetocarmine and glycerine jelly. Such preparations were extremely useful in understanding the morphology of the endosperm.

OBSERVATIONS

Endosperm

The mature embryo-sac is 8-nucleate and contains considerable quantities of starch. The synergids and antipodal cells are generally ephemeral (Text-Fig. 1) but the antipodals sometimes persist during the early stages of endosperm development (Text-Figs. 2, 3, 6). The first



TEXT-FIGS. 1–8. Fig. 1. Double fertilization, ×252. Fig. 2. Two-celled endosperm, ×252. Fig. 3. Endosperm with 2-nucleate micropylar, chalazal and central chambers, ×252. Fig. 4. Both the nuclei in the central chamber in division; note the elongated zygote, ×252. Fig. 5. Nuclear division in the chalazal haustorium; no central chamber is seen, ×252. Figs. 6, 7. Abnormal endosperms, ×252. Fig. 8. Undivided primary endosperm nucleus and a 10-celled proembryo, ×252. (*cc*, central chamber; *ch*, chalazal chamber and haustorium; *emb*, embryo; *mh*, micropylar chamber and haustorium; *pen*, primary endosperm nucleus; *uc*, upper chamber; *z*, zygote).

division of the primary endosperm nucleus is accompanied by a wall which separates a small chalazal chamber (*ch*) from a larger upper

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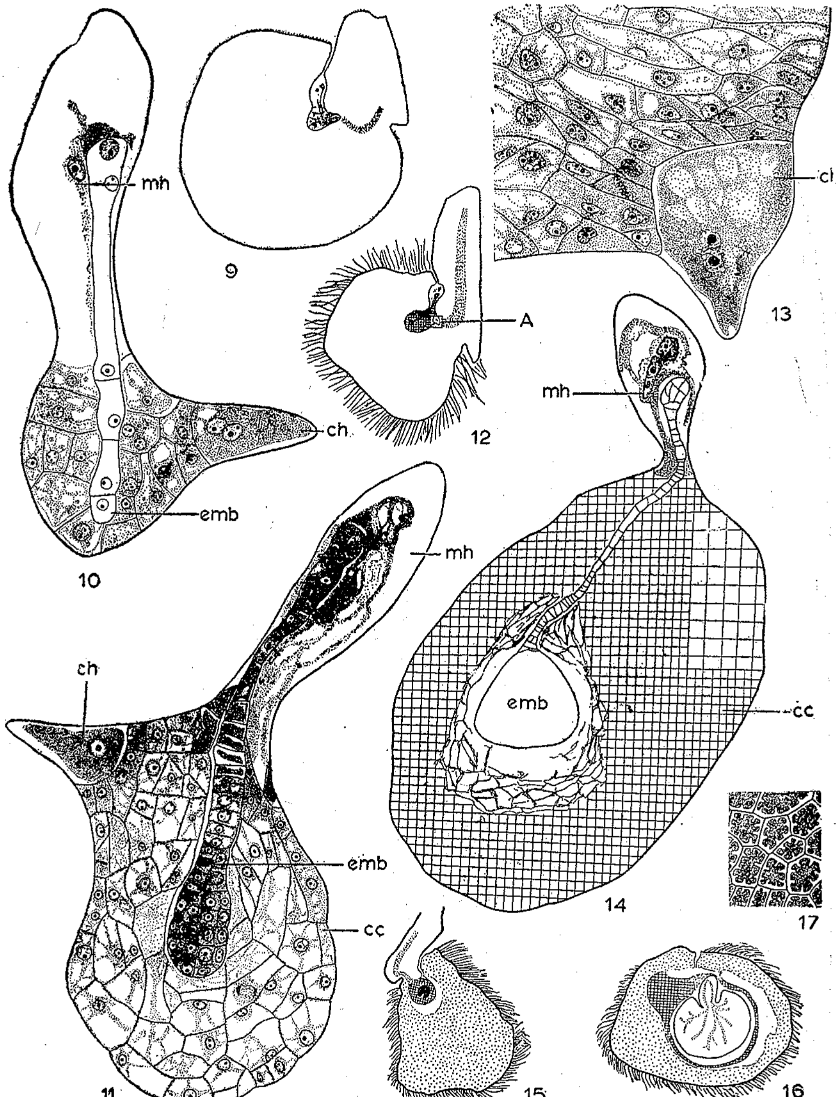


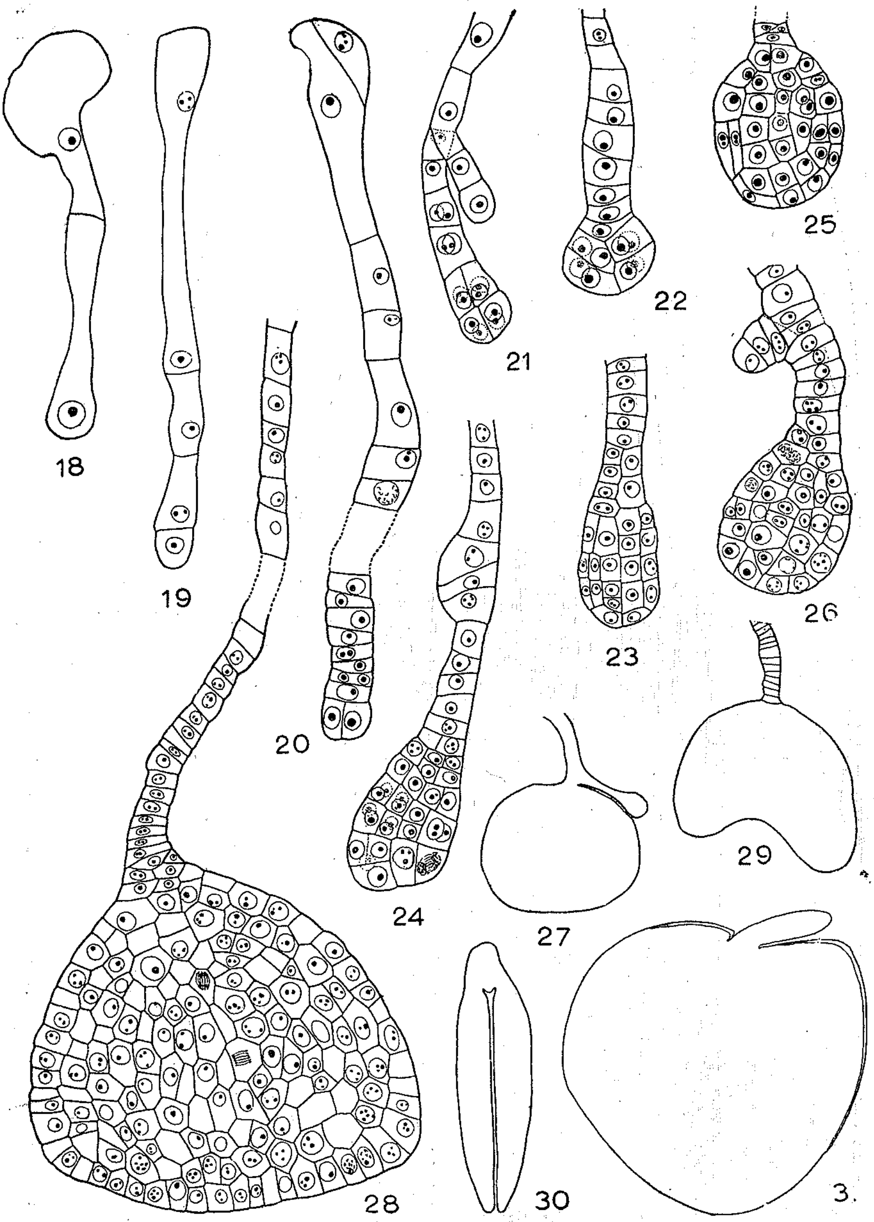
TEXT-FIGS. 9–17. Fig. 9. L.s. ovule at the stage shown in Fig. 10, ×25. Fig. 10. Endosperm with cellular central chamber, ×275. Fig. 11. Endosperm at the early globular stage of the embryo, ×275. Fig. 12. L.s. ovule at the late globular stage of the embryo, ×17. Fig. 13. Enlarged view of the portion marked A in Fig. 12 showing the denser cells near the chalazal haustorium, ×275. Fig. 14. Endosperm with a large central chamber and a micropylar haustorium. The chalazal haustorium has disappeared, ×110. Figs. 15, 16. Longisections of the ovules showing the gradual consumption of the endosperm by the growing embryo, ×8. Fig. 17. Thick-walled cells of mature endosperm in surface view, ×180. (*cc*, central chamber; *ch*, chalazal haustorium; *emb*, embryo; *mh*, micropylar haustorium.)

chamber (*uc*) (Text-Fig. 2). The former soon becomes binucleate and develops into the chalazal haustorium. The upper chamber now segments into a micropylar haustorium and a central chamber (*cc*) (Text-Fig. 3). Thus an initial row of three endosperm chambers is formed. Occasionally, the upper chamber undergoes a nuclear division without cytokinesis. In such cases a micropylar haustorium cannot be delimited (Text-Figs. 5, 6). Text-Figure 7 represents a condition where the primary endosperm nucleus has divided repeatedly to produce a mass of nuclei lying in the chalazal region. Text-Figure 8 shows an abnormal situation where the primary endosperm nucleus has remained undivided, although the embryo has attained a 10-celled stage.

Of the three primary chambers of the endosperm, the middle undergoes several nuclear divisions. At the same time, the chalazal part of the endosperm bends at right angles to the long axis, so that the embryo-sac assumes the shape of a stocking. After nearly 16–32 nuclei have been formed, cytoplasmic furrowing commences and soon the entire central chamber is walled-up (Text-Figs. 9, 10). At first these walls are so fine that one may easily miss them, if the stain is not sharp. Further increase in the endosperm tissue results from non-synchronous divisions of its cells (Text-Fig. 11) and the original shape of the embryo-sac is soon lost by the unequal downward growth of the central endosperm chamber (Text-Fig. 11). The cells of the endosperm which lie in the vicinity of the chalazal haustorium show denser cytoplasm and larger nuclei as compared to the rest of the cells (Text-Figs. 12, 13). Since these cells become larger after the degeneration of the chalazal haustorium, it may be assumed that they take up the role of the chalazal haustorium in supplying nourishment to the endosperm proper. The enlarging embryo consumes the bulk of the surrounding endosperm tissue (Text-Figs. 14–16). The latter, in turn, devours the entire integument save its epidermis. In the mature seed a 3- or 4-layered, thick-walled endosperm persists. In surface view the endosperm cells show characteristic thickenings which intrude into the cell cavity (Text-Fig. 17).

The chalazal haustorium is usually 2-nucleate but sometimes shows a single nucleus (Text-Fig. 11). Being in direct contact with the vascular supply, this haustorium helps in conveying food materials to the endosperm proper. The haustorium shows signs of senescence after the globular stage of the embryo, and by the time a heart-shaped embryo is formed, no trace of it is left in the ovule (Text-Figs. 14, 15).

The micropylar haustorium, contains two hypertrophied and irregular nuclei (Text-Fig. 14). Occasionally one of these may divide resulting in a 3-nucleate condition. Although the wall that separates the central endosperm chamber is destroyed by the elongating pro-embryo, the micropylar haustorium remains as a distinct entity and cells of the endosperm proper do not intrude into it. The micropylar haustorium degenerates at about the same time as the chalazal haustorium.



TEXT-FIGS. 18-31. Figs. 18, 19. Two- and four-celled proembryos, ×275. Fig. 20. Multicelled proembryo, ×275. Figs. 21, 22. Octants; note suspensor budding in Fig. 21, ×275. Figs. 23–25. Stages in the development of the globular embryo, ×275. Figs. 26, 27. Embryos showing budding of suspensor. Fig. 26, ×275. Fig. 27, ×111. Fig. 28. Globular embryo, ×275. Fig. 29. Heart-shaped embryo, ×111. Figs. 30, 31. Mature embryo in vertical section and surface view respectively. The latter clearly indicates the bent radicle, ×7.

Embryo

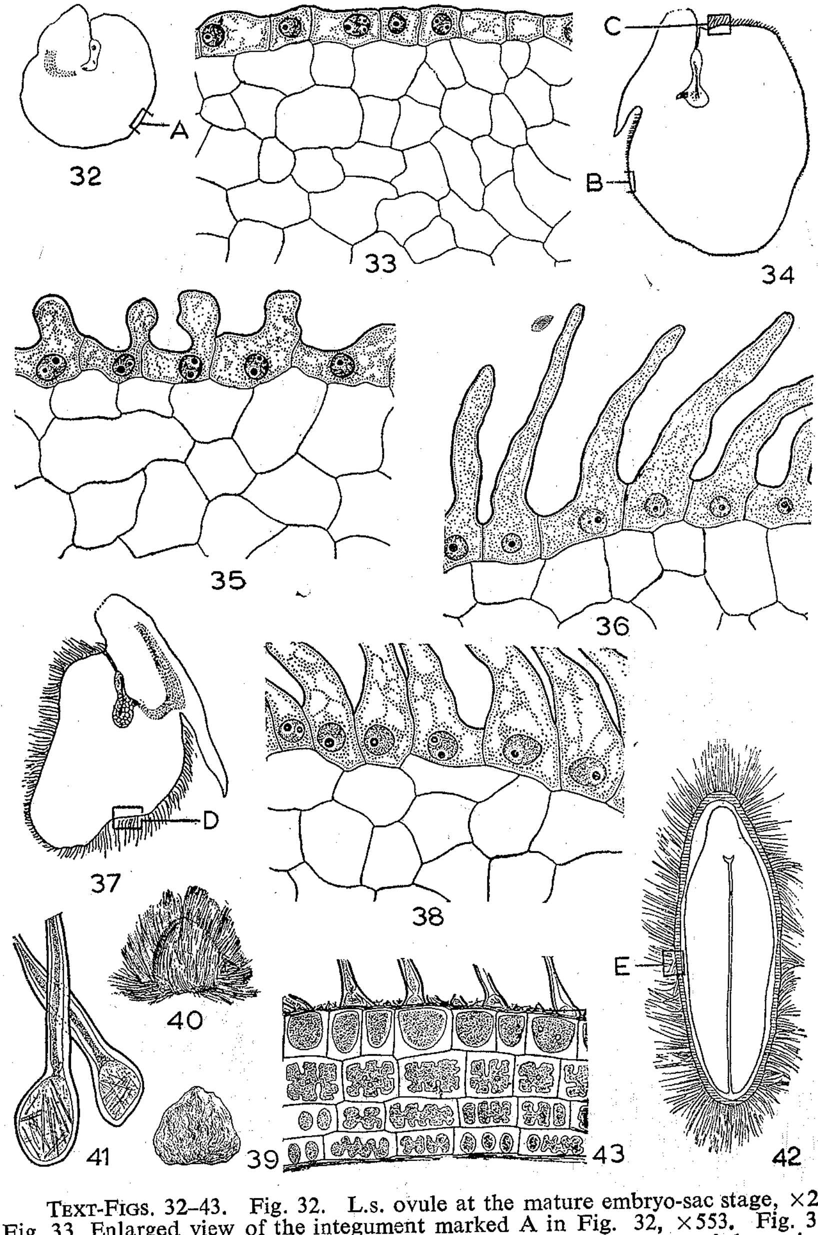
The zygote shows a densely staining collar-like structure round its base (Text-Figs. 1–6), the morphological nature of which could not be ascertained. Prior to division, the zygote elongates considerably. The first division is transverse (Text-Fig. 18) and both the daughter cells divide again in the same plane (Text-Fig. 19). Further transverse divisions produce a long and multicellular proembryo, the terminal cell of which undergoes a vertical division (Text-Fig. 20). The latter, after passing through the octant and globular stages, develops into a dicotyledonous embryo (Text-Figs. 21–31). The suspensor is very long and consists of a single row of cells (Text-Figs. 20, 24, 28), except in older stages when some of the basal cells may divide in vertical plane also (Text-Figs. 11–14). Budding of the suspensor may produce small proembryos which do not usually develop beyond 2–3 celled stages (Text-Figs. 21, 26). However, in one case a sufficiently advanced second embryo was seen (Text-Fig. 27). The mature embryo has large cotyledons with oil as the chief food reserve and a curved radicle (Text-Fig. 31).

Seed

The discoid, anatropous ovules, which are generally two per locule, are attached to the middle of the dissepiment, one above the other. The funiculus is short and the integument which is very massive leaves a narrow but straight micropyle (Text-Fig. 32). The epidermal cells are broader than long and have prominent nuclei (Text-Fig. 33). At the octant stage of the embryo, the ovular epidermis starts elongating in the radial direction and marks the differentiation of the hair initials. Those lying near the micropylar end grow faster than the others (Text-Figs. 34–36). As each hair enlarges in size, its cytoplasm becomes sparser and vacuolation appears all along its length (Text-Figs. 37, 38). During the maturation of the seed the hairs undergo but little change in the thickness of their walls. The dry seed is ovate and dull grey in colour. A number of silky white hairs are closely adpressed to its surface (Text-Fig. 39). On wetting, the hairs absorb water and straighten out within a few minutes (Text-Fig. 40). The embryo is very large and fills the entire cavity of the seed (Text-Fig. 42). Except for the outer epidermis nothing remains of the massive integument. Four layers of endosperm persist bslow it (Text-Fig. 42). The endosperm cells are very thick-walled, the thickenings being thrown into folds. The base of each hair becomes inflated and contains abundant raphides (Text-Fig. 41).

Discussion

The female gametophyte of *Barleria prionitis* (Phatak and Ambegaokar, 1955, 1956) differs from that of *B. cristata* in the persistence and proliferation of the antipodal cells. This feature is a rarity in the Acanthaceae and has been reported only in *Aphelandra aurantiaca* (Hartmann, 1923), *Elytraria acaulis* (Johri and Singh, 1959), *Asystasia gangetica* and *Ecbolium linnaeanum* (Mohan Ram, unpublished). In



TEXT-FIGS. 32–43. Fig. 32. L.s. ovule at the mature embryo-sac stage, ×21. Fig. 33. Enlarged view of the integument marked A in Fig. 32, ×553. Fig. 34. Index sketch for Figs. 35 and 36, ×22. Figs. 35, 36. Magnified view of the regions marked B and C in Fig. 34×553. Fig. 37. L.s. ovule showing well-developed hairs, ×22. Fig. 38. Magnified view of the part of the integument marked **D** in Fig. 37, ×367. Figs. 39, 40. Surface view of a dry seed with adpressed hairs and another which was soaked in water, ×3. Fig. 41. Hairs containing raphides in the swollen basal part, ×240. Fig. 42. V.s. at right angle to the mature seed, ×10. Fig. 43. Magnified sketch of area denoted E in Fig. 42, ×240.

all acanthaceous plants so far investigated, with the exception of *Thunbergia*, the division of the primary endosperm nucleus is accompanied by a transverse wall demarcating a chalazal chamber from the rest of the embryo-sac. The latter undergoes a further transverse partition so that a row of three endosperm chambers is formed. The endosperm proper originating from the central chamber shows a good deal of variation in its mode of development. It may be *ab initio* cellular as in *Acanthus*, *Crossandra* (Mauritzon, 1934), *Barleria prionitis* (Phatak and Ambegaokar, 1956), *Elytraria acaulis* (Johri and Singh, 1959) and *Andrographis serpyllifolia* (Mohan Ram, 1960); or it may be nuclear to begin with and later become cellular as in *Ruellia*, *Asteracantha*, *Justicia* (Mauritzon, 1934) and many other genera. Cell formation is not always complete in the nuclear endosperm and a free nuclear basal apparatus is formed (Mauritzon, 1934; Maheshwari and Negi, 1955; Mohn Ram and Sehgal, 1958). Complete cell formation without a basal apparatus occurs in *Schaueria calycotricha* and *Jacobinia pauciflora* (Mauritzon, 1934). *Barleria cristata* is similar but here the free nuclear stage of the endosperm proper is very short-lived.

The invasion of the micropylar haustorium by the cells of the central chamber, as observed in *B. prionitis* (Phatak and Ambegaokar, 1956), is a unique feature hitherto unknown in any other member of the Acanthaceae. The micropylar chamber in *B. cristata* is invariably coenocytic. In all probability, the so-called cellular endosperm tissue invading the micropylar haustorium is of suspensor (embryonic) origin, for in both *B. prionitis* and *B. cristata* the basal part of the suspensor is known to undergo repeated divisions.

The early embryogeny and occurrence of suspensor embryos are strikingly similar in both the species of *Barleria*. The mature seed of *B. prionitis* (Phatak and Ambegaokar, 1956) has been described as having long hairs along the margins and smaller ones on the surface. In *B. cristata*, however, the hairs are of uniform size all over the seed.

Summary

The female gametophyte is 8-nucleate. The first two divisions of the endosperm are transverse and a row of three chambers is formed. The terminal and basal chambers develop into 2-nucleate haustoria while the middle one alone contributes to the bulk of the endosperm. The early divisions of the endosperm proper are nuclear, but, after about 16–32 nuclei have been produced, furrowing of cytoplasm sets in and the entire endosperm becomes cellular without forming a basal apparatus. In the mature seed four layers of thick-walled endosperm cells persist. The entire integument except the hairy epidermis is used up during the maturation of the seed. The embryo development appears to correspond to the Solanad type. Additional embryos produced by the proliferation of the suspensor have been observed as an abnormality.

Acknowledgements

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* Not seen in original.