FERTILIZATION AND THE DEVELOPMENT OF EMBRYO AND SEED IN *EUPHORBIA HIRTA* LINN.

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In an earlier publication the author in collaboration with Rao (Kajale and Rao, 1943) described the development of the embryo sac and pollen in *Euphorbia hirta* and *Jatropha gossypifolia* with some observations on the organization of the obturator in two more species of the genus *Euphorbia*. In this paper it is proposed to deal with fertilization and the development of the embryo, endosperm and seed coat in *Euphorbia hirta*.

The earlier embryological literature dealing with the Euphorbiaceae is not summarized here since it has already been reviewed by Johri and Kapil (1953), Banerji (1951), Johansen (1950), and Gopinath and Gopalkrishnan (1949).

The material was collected locally and fixed in Navaschin's fluid. It was dehydrated and embedded in paraffin according to the customary methods. Sections were cut 8 to 14 μ thick, stained in Heidenhain's haematoxylin and destained in a saturated solution of picric acid.

FERTILIZATION

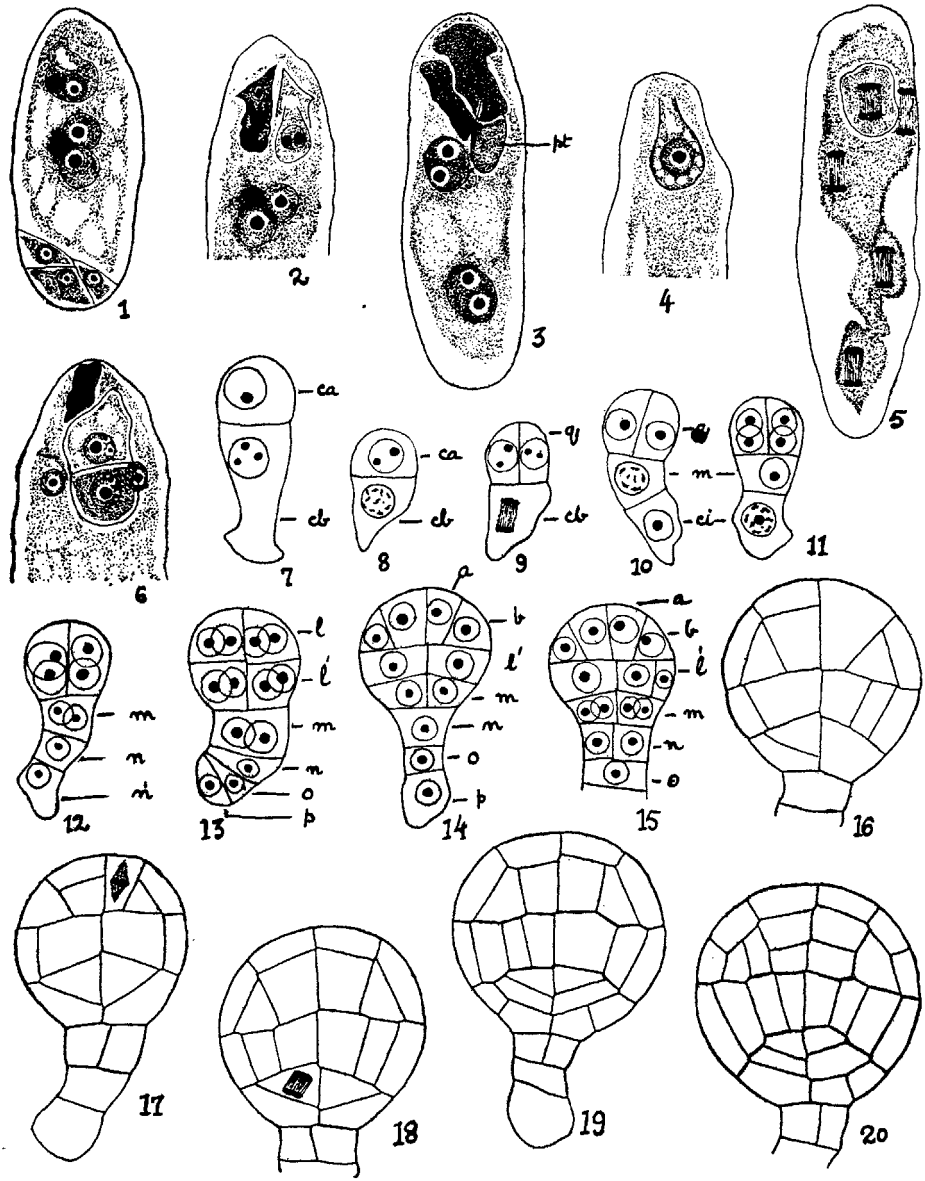
Fertilization is porogamous. After entering the embryo sac the pollen tube passes along one of the synergids, in between the latter and the wall of the embryo sac. It travels up to the base of the egg, its tip enlarges and bursts open discharging the two male gametes inside the embryo sac. During its passage, the pollen tube generally destroys one and sometimes both the synergids (Figs. 2 and 3). Inside the embryo sac the pollen tube may persist till the proembryo becomes two- to four-celled and several endosperm nuclei are formed, but ultimately it collapses and disappears (Fig. 6).

The male gametes at this stage present a spherical shape and take a deep stain. One of them fuses with the egg and the other with the two polar nuclei (Figs. 1 and 2).

During triple fusion the two polar nuclei increase in size and lie, closely pressed together, near the egg apparatus. The male gamete comes in contact with these two nuclei (Figs. 1 and 2). By this time the male gamete in the egg is already in process of fusion to accomplish syngamy (Fig. 2). Further details could not be observed and it could not be ascertained whether there is a formation of the secondary nucleus prior to triple fusion or all the three nuclei fuse simultaneously to form the primary endosperm nucleus. It appears probable, however, that syngamy precedes triple fusion in *E. hirta* unlike in *E. oreophila* and *Homonia retusa* (Gopinath and Gopalkrishnan, 1949) in which syngamy and secondary fertilization are reported to be simultaneous. Johri and Kapil (1953) have recently reported that syngamy precedes fertilization of the fusion nucleus in *Acalypha indica*.

EMBRYO

The fertilized egg becomes somewhat uniformly vacuolate (Fig. 4) and before it divides, four to eight endosperm nuclei are present in the embryo sac (Fig. 5).



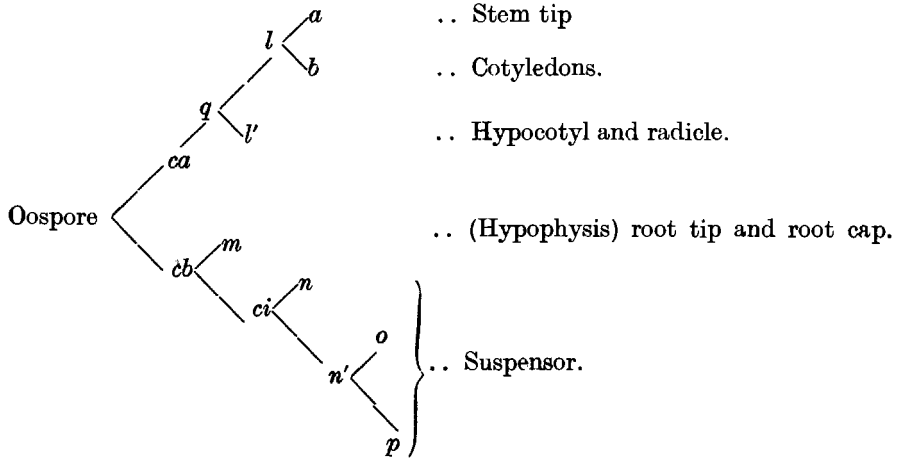
TEXT-FIGS. 1–20. *Euphorbia hirta.*

Fig. 1. Embryo sac showing double fertilization. Note the presence of antipodals. Fig. 2. The same as fig. 1 with one degenerating synergid. Figs. 3 and 4. Successive sections of the same embryo sac; fig. 3 shows two degenerating synergids, the tip of the pollen tube (*pt*) and two endosperm nuclei, while fig. 4 shows the micropylar part of embryo sac with the fertilized egg. Fig. 5. Embryo sac showing division of the oospore and endosperm nuclei. Fig. 6. Micropylar part of the embryo sac with two-celled proembryo and degenerating pollen tube. Figs. 7 to 20. Various stages in the development of the embryo. ×600.

The first division of the oospore is transverse and results in the formation of two cells, the terminal cell and the basal cell (Figs. 5, 7 and 8). These cells are designated by the letters *ca* and *cb* respectively. Generally the first division of the oospore in the Euphorbiaceae is transverse but it is claimed to be longitudinal in *E. preslii* (Weniger, 1917) and *E. rothiana* (Shrivastava, 1952).

The basal cell *cb* varies in shape and size. Generally it is larger and more vacuolate than the terminal cell *ca*. Sometimes it is elongated (Fig. 7). The basal cell *cb* divides transversely either before or after the terminal cell *ca* has divided longitudinally and gives rise to the upper cell *m* and the lower cell *ci*. At this stage the proembryo is T-shaped and consists of three or four cells (Figs. 9 and 10). The cell *ci* divides further and forms the cells *n* and *n'* (Fig. 12). Normally the division of the cell *ci* is in a transverse plane but occasionally it may be oblique or nearly vertical. The cell *n'* by a further transverse division gives rise to two cells, *o* and *p* (Figs. 13 and 14). Thus *m*, *n*, *o* and *p* are all derived from the basal cell of the two-celled proembryo.

The terminal cell *ca* divides longitudinally to form two juxtaposed cells designated together as *q* (Fig. 10). Both these cells divide again by a longitudinal wall at right angles to the first and a quadrant is formed (Figs. 11 and 12). Shortly thereafter each cell of the quadrant divides transversely into two tiers *l* and *l'* and the octant stage is reached (Fig. 13). The upper four cells of the octant eventually give rise to the stem tip and the two cotyledons, and the lower four cells to the hypocotyl. The cell *m* derived from the basal cell (*cb*) functions as an hypophysis which completes the root cap and the root tip. The remaining cells *n*, *o*, *p* and their derivatives develop into the suspensor. The origin of the various parts of the embryo from the cells of the proembryo is as follows:—



Thus the embryogeny of *E. hirta* in all essential features resembles that of *E. exigua* described by Souèges (1925) and conforms to the *Euphorbia* variation of the Onagrad Type (Johansen, 1950) or to the Crucifer Type of Maheshwari (1950). Similarly, according to Johansen (1950), the development of the embryo in *E. procera* (Modilewski, 1909), *E. splendens* (Weniger, 1917), *E. esula* (Souèges, 1924) and *Acalypha australis* * (Tateishi, 1927) also conforms to the *Euphorbia* variation. Two other variations have also been reported from this family. In *E. preslii* (Weniger, 1917) and *E. rothiana* (Shrivastava, 1952) the embryo development

* According to Johri and Kapil (1953) the embryo development in this species is like that of *A. indica*.

conforms to the *Scabiosa* variation of the Piperad type (Johansen, 1950) while in *Acalypha indica* (Johri and Kapil, 1953) and *A. lanceolata* * (Thathachar, 1952) it conforms to the *Lotus* variation under the Onagrad Type (Johansen, 1950).

Details of the development of the various parts of the mature embryo are discussed in the following paragraphs.

At about the time when the octants are formed, the embryo consists of six tiers: the two terminal tiers *l* and *l'* of four cells each, the next one *m* of two cells, and the lower three tiers, i.e , *n*, *o* and *p* consist of one cell each (Fig. 13). Each cell of the terminal tier *l* divides obliquely and gives rise to two cells, *a* and *b* (Fig. 14). The inner cells *a* by transverse and longitudinal divisions give rise to the plumule, while the outer cells *b*, two on each side, form the initials of the cotyledons (Figs. 20 to 22). Simultaneously with this development the cells of the tier *l'* divide periclinally to cut off the dermatogen (Fig. 15). Shortly afterwards the dermatogen is completed in the other two tiers *l* and *m* also (Figs. 16 and 17). The differentiation of the dermatogen in the tier *l* does not follow any regular sequence and it may be completed first either in the inner cells *a* or the outer cells *b* (Figs. 16 and 17).

The cells of the tier *l'* at first divide longitudinally and then undergo a series of longitudinal and transverse divisions so as to form the region of the hypocotyl and radicle (Figs. 18 to 21). As the development proceeds the periblem and the plerome become gradually differentiated. At about the stage represented by Fig. 22 the plerome consists of five to six layers and the periblem of three to four layers as seen in longitudinal sections.

It has already been stated that the cell *m* derived from the basal cell (*cb*) of the two-celled proembryo functions as an hypophysis. Before the octant is organized, it divides by a vertical wall and gives rise to two cells which in turn also divide vertically to form a group of four cells (Figs. 12 to 15). All these cells divide periclinally to produce four outer cells and four inner cells (Fig. 16). The former complete the dermatogen and represent the primordium of the root cap. The inner cells once more divide obliquely and the resulting eight cells are placed in two tiers of four cells each (Figs. 18 to 20). These two tiers function as the initials of the root cortex and form the root tip. The upper four cells contribute to the plerome while the lower four form the periblem (Fig. 22).

Suspensor

Except the cell *m*, the remaining cells *n*, *o* and *p* derived from the basal cell (*cb*) give rise to the suspensor. It consists of a few cells and becomes multiseriate especially towards the embryo side (Fig. 21), and persists even in the mature seed. Its cells become packed with starch grains during later stages of development. The absence of the suspensor in this family has been previously reported by Weniger (1917) in *E. preslii* and Shrivastava (1952) in *E. rothiana*.

Mature Embryo

The mature embryo is straight and dicotyledonous (Fig. 23). The three histogenic layers become prominent at about the stage shown by Fig. 22. The cells of the periblem are broader than long. In the region of the plerome they are more elongated. The cells of the mature embryo become packed with food material mostly starch grains. Their distribution, however, in the different histogenic layers is not uniform. More grains are deposited in the periblem than in the plerome. In the periblem, they are also bigger in size than those in the plerome. An interesting point about the structure of the embryo is the development of the

* See discussion by Johri and Kapil (1953).

long, elongated and narrow laticiferous cells which are densely cytoplasmic. They are present in the hypocotyl and the cotyledons.

Endosperm

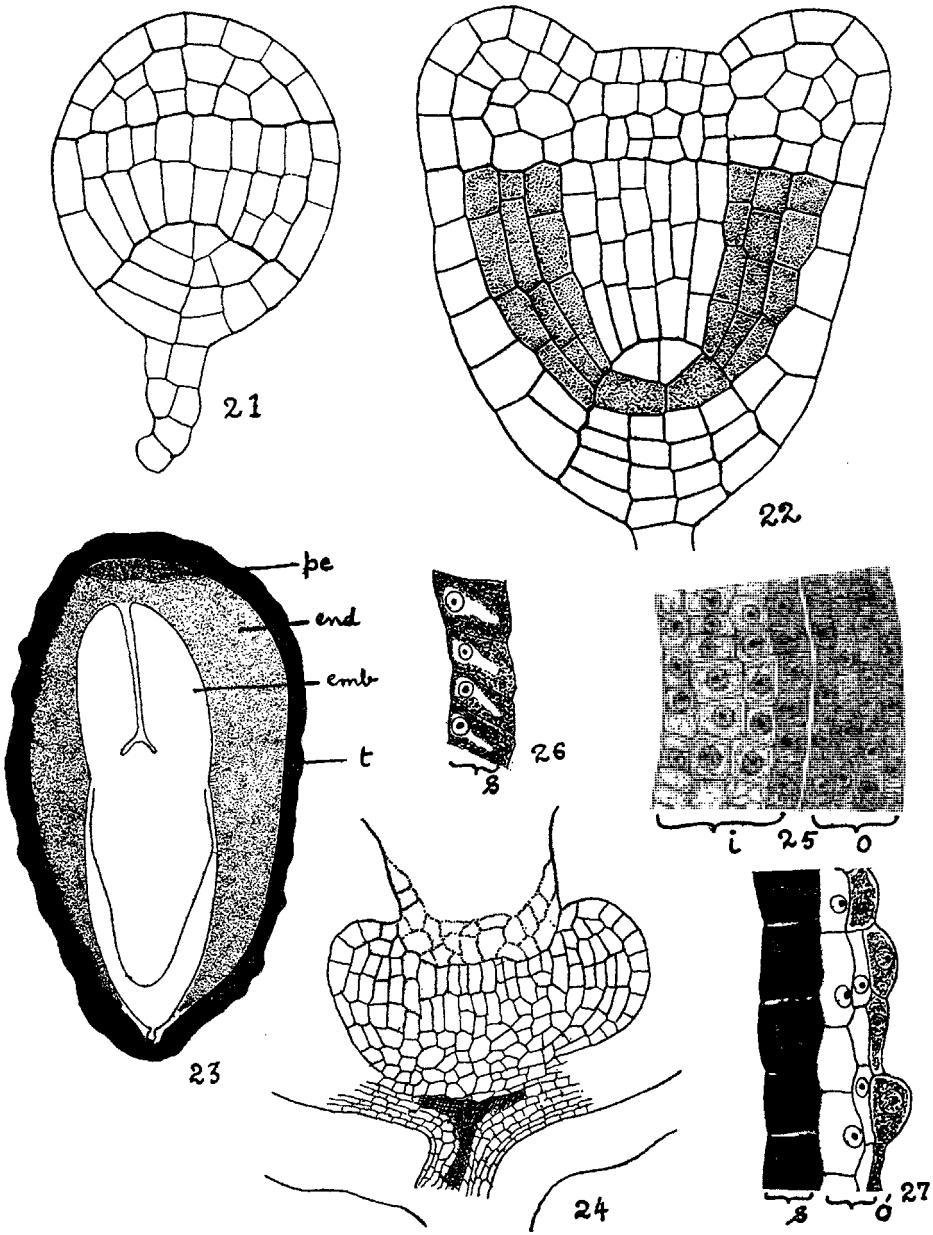
The primary endosperm nucleus divides earlier than the oospore and forms two free nuclei. One of them moves towards the chalazal end and soon both of them divide to form four to eight nuclei before the oospore segments transversely (Figs. 3 and 5). In *E. splendens* (Weniger, 1917) as many as 32 to 64 endosperm nuclei are formed before the division of the oospore. The free nuclear division in the endosperm continues until many nuclei are formed in the peripheral layer of the cytoplasm. They are somewhat evenly distributed except at the two poles of the embryo sac where they are more crowded. During earlier stages of development, the divisions of the endosperm nuclei are synchronous but later on the nuclei are in different stages of division. The endosperm nuclei are further characterized by the presence of a varying number of nucleoli as reported by Modilewski (1909, 1911) in *E. procera* and *E. palustris*. In *E. hirta* the nucleoli vary from one to four in number. The free nuclear endosperm later becomes cellular. Wall formation starts all round at the periphery and extends towards the centre. The process of wall formation begins before the embryo becomes cordate-shaped and after the differentiation of the dermatogen in the octant.

In the beginning the cells of the endosperm are highly vacuolate but soon they become filled with cytoplasm and appear more compact and conspicuous. Such a change occurs first in the cells at the two poles of the embryo sac. In the mature seed the cells of the endosperm are loaded with starch grains. The latter are bigger in size than those in the embryo. A part of the endosperm is consumed by the growing embryo but a considerable part of it persists in the mature seed surrounding the embryo except at the tip of the root cap (Fig. 23). A part of the nucellus, described later, also persists in the mature seed at the chalazal end along with the endosperm (Fig. 23).

Development of the Hypostase

Some other interesting changes also occur during post-fertilization stages which deserve consideration. At the chalazal end the embryo sac elongates and destroys some of the cells of the nucellus. The remaining cells, however, become rich in cytoplasm, take a deeper stain and appear more regularly placed. They multiply and become conspicuously differentiated to form a thick saucer-shaped pad of tissue below the chalazal end of the embryo sac, the further growth of which is arrested as soon as it reaches this pad (Fig. 24). Afterwards, however, the embryo sac extends laterally above the pad and eventually destroys all the adjoining cells of the nucellus. The formation of such a pad is analogous to the hypostase and its occurrence is also reported by Gopinath and Gopalkrishnan (1949) in *E. oreophila* and by Landes (1946) in *Acalypha rhomboidea* and some other species of the genus *Euphorbia* investigated by her. The vascular supply of the ovule ends below the hypostase, which persists in the mature seed as perisperm. Like the endosperm it functions as an organ of storage and its cells become packed with starch grains as the seed matures (Fig. 23). The function of this tissue is a matter of dispute. Either it is considered to be of nutritional nature or it is supposed to arrest the downward growth of the embryo sac. Whatever be its earlier function, in later stages at least it is definitely an organ of storage in *E. hirta*.

An interesting change occurs in the structure of the nucellus during the formation of the hypostase. As reported by Landes (1946) for other species of *Euphorbia*, the cells of the inner integument at the chalazal end become enlarged at the expense of the nucellus and divide the latter into a bigger micropylar portion and a smaller chalazal portion. The former consists of a few layers of large and vacuolate cells



TEXT-FIGS. 21–27. *Euphorbia hirta.*

Figs. 21 and 22. Stages in development of embryo. ×600. Fig. 23. L.S. of mature seed showing embryo, endosperm, persisting hypostase and seed coat. Note the absence of endosperm at the tip of the root cap. ×60. *emb* = embryo, *end* = endosperm, *pe* = perisperm, *t* = testa. Fig. 24. Chalazal part of the ovule showing the hypostase. Note constriction above the hypostase caused by the inner integument. Cells shown by dotted lines represent the degenerating cells of the nucellus. ×260. Figs. 25 to 27. Stages in the development of testa. Fig. 26 shows a few cells of the stony layer only. ×600. *i* = inner integument, *o* = outer integument, *o'* = part of the testa derived from the outer integument, *s* = stony layer.

while the latter consists of compact, regular and densely cytoplasmic cells which later develop into the hypostase. The division of the nucellus into two such portions thus appears to be a characteristic feature of the genus *Euphorbia*.

Seed Coat

There are two integuments in *E. hirta*, the outer consisting of four layers and the inner of four to five layers of cells over the greater part of their length. But at the tip the outer integument is thicker while the inner one is thinner. The latter sometimes consists of six to seven layers of cells towards the chalazal end. In general the cells of the outer integument are richer in cytoplasm than those of the inner integument except the outer epidermal layer of the latter, the cells of which are densely cytoplasmic as those of the outer integument (Fig. 25). As the embryo develops the integuments close over the nucellus and the nucellar beak disintegrates.

Both the integuments take part in the formation of the testa. In the mature seed it consists generally of four and occasionally of three layers of cells. Out of these four layers, the innermost layer of the testa represents the outer epidermis of the inner integument while the remaining two or three layers belong to the outer integument. The outer epidermal layer of the inner integument finally becomes most conspicuous and forms the brittle stony layer of the seed coat as reported by Netolitzky (1926) and Landes (1946) for many other members of the Euphorbiaceae.

The remaining part of both the integuments comprising the inner layers of the inner integument and the inner layer of the outer integument disappears during the differentiation of the testa. Before they disappear their cells increase in size and become vacuolate and those of the inner integument may also multiply to increase the number of layers temporarily.

During maturation of the seed the cell walls of the brittle stony layer become thickened due to the deposition of a chemical substance which looks yellow or yellowish brown when stained with haematoxylin. The thickening matter is first deposited on the radial and the outer tangential walls (Fig. 26). It later extends to the inner tangential walls of the cells. During this time the nucleus shifts to the inner tangential wall and the cytoplasm is gradually reduced. By the time the cytoplasm is completely used up the thickening has progressed centripetally to meet in the middle along an irregular line to form fully thickened stony cells (Fig. 27). The nucleus in these cells is seen to persist for a considerable time but later on it is completely enclosed by the thickening matter and is therefore not visible. The thickening of the cell walls starts at the micropylar end and extends towards the chalaza.

Majority of the cells of the epidermal layer of the testa increase in size and become dome-shaped as a result of which the testa presents a finely warty appearance (Fig. 27). The epidermal cells contain in them the granular deposit of the same substance present in the cell walls of the brittle stony layer (Fig. 27). The nucleus persists in these cells also.

The cells of the middle two layers are without any granular deposits. Out of these two layers, the cells of the outer one are narrow and elongated while those of the inner one are somewhat oblong and bigger than those in the outer layer. The cells in both the layers are vacuolate and show the presence of nuclei in them (Fig. 27).

Summary

Fertilization is porogamous. The pollen tube passes in between the wall of the embryo sac and one of the synergids. Generally one and sometimes both the synergids are destroyed. The polar nuclei increase in size prior to triple fusion. Probably syngamy precedes triple fusion.

The proembryo consists of three to four cells and is T-shaped. Embryo development conforms to the *Euphorbia* variation of the Onagrad Type. The small suspensor is multiseriate

towards the embryo side and persists in the mature seed. The structure of the mature embryo and the distribution of starch are described.

The primary endosperm nucleus divides earlier than the oospore. The free nuclear endosperm later becomes cellular. Wall formation starts at the periphery and extends towards the centre. One to four nucleoli are present in the nuclei of the endosperm cells. The endosperm surrounds the embryo except at the tip of the root cap.

The hypostase persists in the mature seed as perisperm and serves as an organ of storage.

The testa consists of four layers of cells. The innermost layer representing the outer epidermis of the inner integument becomes brittle and stony. Next come three more layers derived from the outer integument. Granular deposits are present in the epidermal cells of the testa which have a finely warty appearance. There is a difference in the size and shape of the cells of the different layers.

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