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# EMBRYOLOGICAL STUDIES IN *ORYZA SATIVA* L.

By

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(With Plates VI—IX)

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## Introduction

Many investigations on rice, the most important crop plant of our country, have been undertaken of late from different standpoints, but not much research has been done on the development of the embryo sac, including the development of the embryo and endosperm. So far as the author is aware, only two papers have been published, namely the cytological study on the development of the embryo sac and the formation of the endosperm by Y. KUWADA (1910), and the investigation, among others, carried out mainly on the development of the embryo by M. AKEMINE (1914).

The purpose of this paper is to report a study of the development of the embryo and endosperm from the stage of the first appearance of ovular tissue up to the almost perfect formation of them.

I shall be satisfied if this report furnishes any information to those who are engaged in research work on plant breeding, agronomy, or botany.

## Material and Methods

As the process of the formation of the embryo and endosperm may be different in different varieties of rice, only one variety *Nioiwase*, cultivated in the Experimental Rice Field of the Hokkaido Imperial University, was taken as the material for my investigation.

To examine the development of embryo sac and the phenomenon of fertilization, ovaries were taken from panicles, 6, 10, 16, 18 and 20 cm. in length respectively, from the spikelets in anthesis, and also from spikelets 4–18 hours after anthesis (at intervals of two hours generally). All materials

were fixed in Flemming's solution (Bonner Gemisch). To examine the development of embryo and endosperm the author fixed the ovaries of different ages after the anthesis of their spikelets in the same solution. But when old ovaries and nearly perfectly matured embryos separated from the ovaries were examined, formalin acetic alcohol (5 cc. glacial acetic acid and 5 cc. commercial formalin added to 90 cc. 50 per cent alcohol.) was used as a fixative, and turpentine oil was used as a clearing agent instead of chloroform as in other cases.

For staining, Heidenhain's iron-alum haematoxylin was used and the microtome sections were made 8-20  $\mu$  thick.

As a pollen tube stains dark or gives dark staining granules or fragments in haematoxylin at about the time of its entrance into the embryo sac or of the fertilization, it is very hard to examine the male nuclei discharged out of pollen tube. Accordingly the author used the combination stain of cyanin and erythrosin recommended by Miss THOMAS, which is said to stain the male nuclei blue and the female red, but the author didn't succeed even with this.

To examine the deposition of starch grains, the iodine potassium-iodide solution (1 gr. iodine added to 100 cc. of 5 per cent potassium iodide solution) was used.

All drawings in this paper were made with the aid of a camera lucida, and the figures were drawn from single fields of vision if possible.

### The Development of the Embryo Sac

In *Oryza sativa* almost the same process of embryo sac development can be found as in other forms of Poaceae such as wheat (PERCIVAL 1921, WATKINS 1925) and *Avena sativa* (TANNERT 1905).

As shown in fig. 1, the matured megaspore mother-cell is somewhat trapezoidal in shape, and two or three times larger than other cells in the nucellus. It occupies the apex of the axial row of four or five cells immediately beneath the epidermis. After this megaspore mother-cell is divided into two cells (fig. 2)\*, each is divided again in a similar manner, thus a tetrad row of megaspores is produced (fig. 4). But the dyad does not divide always at the same time, so a triad row of cells is sometimes produced. At this time the uppermost cell stains deeply, indicating its lapsing into the degeneration stage (fig. 3).

The outer three cells of a tetrad row slowly collapse and degenerate,

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\*Y. KUWADA has observed the meiotic division occurring at this time.

while the innermost cell survives and gradually enlarges itself (fig. 5), forming at last an embryo sac.

The nuclear division of the innermost megaspore soon occurs and two nuclei are formed quite apart from each other. One of the two nuclei thus produced occupies the original subepidermal position of the degenerated three sister cells, pushing apart the cells on the sides of the narrow channel left by the degenerated cells (fig. 6). The distance between these two nuclei is about  $41 \mu$ , but they are connected with each other by light staining cytoplasm, and enclosed within a well stained membrane.

Fig. 7 shows the four-nucleated embryo sac, two nuclei each at the micropylar as well as at the opposite end which are derived from the mother nucleus each in the corresponding position.

As the embryo sac grows, the nucellar cells around it seem to be disintegrated, finally to be absorbed, by it. Their debris is shown also in fig. 7 around the pear-shaped embryo sac, which has its base on the micropylar side. Through the further division of each nucleus in the same sac, there come to exist two masses of nuclei, each consisting of four nuclei.

Fig. 8 shows four nuclei in a group at the antipodal region, and fig. 9 three nuclei in a group at the micropylar end, together with one nucleus moving from its position towards the central region. Not only from the micropylar but also from the antipodal region of the embryo sac, one of the four nuclei moves up to the central region. These two nuclei which have migrated to the central region come in contact with each other without fusing together and become polar nuclei. They continue this contact condition until the triple fusion occurs. At the time when the polar nuclei, each having a large nucleolus of about equal size, have come in contact, a few nuclei, or those with their obscure membranes, or a few small nucleoli—chromatic bodies which might have been derived from the primary nucleus by tearing themselves off (fig. 10),—appear in each antipodal cell (though not always in each). Nearly at this time cell division into several cells, and cell growth follow.

The process above mentioned takes place before heading. At the latest at about the time of anthesis, we can see distinct differences between synergids and egg cell in the size as well as form of their cells and nuclei. By this time the antipodals generally have divided themselves into several cells, usually not more than ten.

Before fertilization, beside the increase in size of each cell in the embryo sac, the contact of the polar nuclei becomes closer, as if the two hemispheres were touching on their flat surfaces (fig. 12). These nuclei

themselves also increase in size as the egg nucleus does, and the antipodals appear on the dorsal side in the upper part of the embryo sac through the growth of it, instead of at the end of the embryo sac (as in figs. 29 and 40). The distance between polar nucleus and egg cell becomes smaller (fig. 11).

Very rarely the author noticed polar nuclei, one of which has two nucleoli (fig. 22).

### Fertilization and Synergids

The pollen tube coming down through the cavity of the ovary along the coat of the ovule after passing through the conductive tissue of the stigma and style, finds its way to the micropyle. It passes through the micropyle by twisting itself and works its way between the cells of the nucellus and as soon as it comes into the embryo sac, its tip swells out by the side of one of synergids.

As shown in fig. 13, the comparatively homogeneous staining of the pollen tube, which makes the examination of its nuclei hard, and the non-destruction of its tip, tells us that its content has not yet been discharged, and here the polar nuclei are in contact with each other, each having a large nucleolus of about equal size.

In one specimen we can see the pollen tube, the tip of which swells out in a similar way to the one shown in fig. 13, but it has been more or less destroyed. In the same specimen the author observed an elongated spiral body at the end of the egg cell, which looks as if consisting of two threads (fig. 14). Probably this body may represent the male nucleus. Besides this, we see the polar nuclei, not yet fused together, which possess more or less differentiated chromatin (fig. 16).

Though the author has not been able to see the second male nucleus discharged from the pollen tube, he can see, within the entirely fused polar nucleus, a nucleus which has a slightly smaller nucleolus than that of the polar nucleus (fig. 15). This figure seems to indicate the triple fusion.

About the time when a few or rarely several endosperm nuclei are formed, we can see in some specimens the egg nucleus having two nucleoli of about equal size, rather smaller than the nucleolus existing alone in an egg nucleus after the division of the primary endosperm nucleus (fig. 17). The author considers that these nuclei with two nucleoli indicate the completion of the fertilization. The appearance of two nucleoli in the fertilized egg nucleus has been observed by a few investigators so far as the

author is aware, namely by PERCIVAL (1921) on wheat, (according to whose report the size of one nucleolus was about half that of the other), by ONO (1926) on *Heloniopsis breviscapa*, and by TANNERT (1905) on *Avena sativa*.

Though it has not been possible for the author to examine the fertilization of egg nucleus definitely, the fertilized egg nucleus seems to be at rest for a while, and after the chromatin appears more densely and distinctly, the egg nucleus enters into the metaphase of the first division, losing its nuclear membrane (figs. 18 and 19). By this time the primary endosperm nucleus completes at least several successive divisions as many investigators have observed in Poaceae. The time taken from anthesis to fertilization (the pollination occurs at the same time as anthesis or a little earlier under normal conditions), though subject to fluctuations, is generally twelve hours, as AKEMINE (1914) has observed.

In a word, the division of the primary endosperm nucleus occurs soon after the triple fusion. Moreover the successive divisions of the endosperm nucleus take place very rapidly.

In general, synergids begin to degenerate before fertilization and their debris remains for a while, sometimes until the fertilized egg cell has been divided into several cells.

### The Development of the Ovule

The ovary in longitudinal section at first takes a "V" shape, at the bottom of which lies a protuberance of meristematic cells larger than those of the other part, with a slight curvature under the epidermis which surrounds the margin of the ovary (fig. 23). This cellular mass is the rudimentary nucellus.

As this protuberance grows both ends of the "V" shaped ovary come nearer to each other and at last make the cavity of the ovary (fig. 24). The lateral position of the nucellar protuberance is probably due to the rapid growth of one side of the ovular tissue.

Soon there develops an inner integument around the base of a nucellus. Perhaps the megaspore mother-cell appears at this stage or a little earlier (fig. 25). At this stage the inclination of the ovule to the longitudinal axis of the ovary is about 70 degrees. When the outer integument appears around the outside of the inner one, the ovule bends 90 degrees or more, and the cellular arrangement of the integuments on the side towards the raphe is thinner than that of those opposite it (fig. 26 which is the same section as fig. 1). As shown in fig. 1, it is common that each in-

tegument consists of three or four cell-layers. Generally in the younger ovaries, the inner integument possesses one cell-layer inserted within the two cell-layers which develop parallel with each other, and the outer integument, two cell-layers. These inserted cell-layers usually collapse into thinner cell-layers. But in the inner integument after heading this inserted cell-layer is not observed generally.

At about the time of the formation of the linear tetrad of megaspores or the enlargement of the innermost megaspore, the inner integument surrounds the nucellus so completely as to make it hard to find the micropyle, but the growth of the outer integument is meager, especially the part opposite the raphe does not reach half way to the micropyle, and even afterwards it does not elongate to this part. At these stages the ovule inclines about 130 degrees (fig. 27).

At about the time when the gametophytic contents of the embryo sac have settled, the cavity of the ovary is filled completely in accordance with the growth of the ovule (fig. 28). Though the ovule of spikelets in anthesis or in a very early stage of endosperm development, bends more or less resulting in a slightly campylotropous ovule, the ovule at these stages inclines 140-155 degrees (fig. 29), and in the later stages of endosperm development it becomes nearly parallel to the longitudinal axis of the ovary.

In general, at the first appearance of ovular tissue, though the megaspore mother-cell and the inner integument do not yet appear, the axis of the ovule nearly coincides with that of the ovary, and as the integuments grow, the inclination of the ovule increases and when the endosperm tissue develops about three layers thick, the ovule becomes anatropous.

Both integuments on the side towards the raphe and the outer one opposite it develop meagerly as compared with the inner one opposite it. Of course in an anatropous ovule there is no need of the longer development of the integuments on the side towards the raphe in an ecological sense. The meager development of the outer integument can be seen clearly by observing cross sections of ovaries at different stages. That is to say, the inner integument covers the nucellus completely, but the outer integument encloses only about two thirds of the inner one on the dorsal side of the ovule, even in its maximum growth (fig. 40).

The degenerating process of the outer integument seems to begin at about the time of anthesis and after a few days its cells are disorganised. The outer layer of the inner integument becomes hardly recognizable, resulting in a very light thin layer about 8 days after anthesis.

By the growth of the embryo sac, nucellar cells around the embryo

sac are stretched out and become scanty in cytoplasm. When the endosperm tissue has become three or four layers thick or the embryo sac is nearly filled up with endosperm, the nucellar cells decay and disappear. Even the epidermis of nucellus which has continuously lived for a long time seems to have lost its vital function notwithstanding its regular arrangement. This nucellar epidermis can be seen even in a milk-ripened grain. Accordingly, the two distinct cell layers between the tube cells and the aleurone layer in the milk-ripe stage are the inner layer of the inner integument and the epidermis of the nucellus, as I. NAGAI (1916) has observed (fig. 44).

### The Formation of the Endosperm

The two nuclei derived from the primary endosperm nucleus generally occupy their positions in the cytoplasm which lies along the marginal part of the embryo sac. They stand fairly far from each other, and are enclosed with thick cytoplasm without forming cell walls.

We can see the two endosperm nuclei in the prophase derived from the primary nucleus. In one of them the chromatin threads loosely group into three lumps, two of which are seen each surrounding a nucleolus of different size, and in the other also the chromatin makes three lumps, each surrounding a nucleolus of about equal size (figs. 30 and 31). But the author has had few chances to observe the nuclei which have three lumps of chromatin threads in this phase. Only the nucleoli always appear distinctly and their numbers are not always three. Frequently two nucleoli, a large and a small, or a large one, or very rarely one large and several small ones make their appearance. In the later stage of endosperm development a large nucleolus is usually to be found while the appearance of two or three nucleoli is not frequent.

In the careful cytological study of endosperm in this plant KUWADA finds that in the telophase of this division the lumps of chromosomes (though not always three) are scattered about the surface of the nuclear cavity and by this time three large nucleoli always make their appearance among them, two nucleoli unite at first to form one and then the third comes to be united with the latter. The author can see also in the later telophase the figures which coincide substantially with KUWADA's observation. But in the author's case, as combination staining had not been used, nucleoli could not be distinguished from sticky lumps of chromatin (figs. 32-34).

These phenomena that triploid endosperm nuclei possess three nucleoli

of a few sticky lumps of chromatin and that fertilized egg nuclei often show two nucleoli, indicate the autonomy of fused nuclei in their fused product,—fertilized egg and endosperm nuclei.

The nuclear division of endosperm nuclei occurs very rapidly and these nuclei distribute at first loosely on the periphery of the embryo sac and later they distribute gradually and more densely in one layer around the embryo sac, as well as around the embryo where they are arranged more densely than in any other region (fig. 37).

In the surface view of cytoplasmic layer, wherein endosperm nuclei in their mitosis are imbedded, we see homogeneously scattered protoplasmic granules, whereas in a layer consisting of resting nuclei, the more differentiated cytoplasmic threads radiate from the cytoplasm which surrounds the endosperm nuclei (figs. 35 and 36).

The process of division of endosperm nuclei is very regular. The second layer of endosperm develops inside the first and so forth centripetally, though the cell layers are thicker in the region near the two ends of the embryo sac than in other region.

It was observed in 1926 that in the ovules seven days after anthesis, two or three layers of endosperm cells have developed, each of them having a cell wall (fig. 45). KUWADA states that in rice the wall formation begins after the embryo sac has been lined with free nuclei. PERCIVAL observes in wheat, that walls between the endosperm cells appear first in the narrow pocket-like cavity in which the embryo lies. Of course, endosperm cells furnished with the walls increase further by mitotic division.

The development of the endosperm is much influenced by environmental conditions. For instance, the above mentioned embryo sac seven days after anthesis is at a far younger stage of development in comparison with that observed about four days after anthesis in 1925, in which the whole cavity was filled up with endosperm cells except the central part. The same is true in the development of embryo, the embryos two days after anthesis in 1925 correspond on the whole in their development to those three days after anthesis in 1926. Even in the same year, according to the period of collecting the materials, the development of embryo and endosperm does not necessarily correspond with the advancement of date of collection.

Gradually even the interior of the embryo sac becomes filled up with endosperm cells, small cells at the peripheral region compactly placed, and large ones inwards loosely (figs. 42 and 44), but those around the embryo are obliterated by the growth of the latter (fig. 42).

The storage of carbohydrates in the endosperm tissue seems to begin when it has nearly filled the embryo sac cavity, about 8-10 days after anthesis (the ovary in this stage shown in fig. 42). Treating these samples with iodine potassium-iodide solution, the middle part of endosperm tissue which comprises the greater part of the ovary, stains reddish brown more deeply than the upper part, (morphological base in an anatropous ovule) but the terminal end of the endosperm tissue stains only a little. The lower part where the embryo lies and also the outermost peripheral layer of endosperm tissue (the rudimentary aleurone layer) remains yellowish.

When examined under the higher powered microscope, those substances stained reddish brown are almost oval in shape. They are as large as the nucleus of the endosperm, are stained heterogeneously and gather around the nuclei. Perhaps these substances are composed of dextrin.

The fact that the endosperm tissue near the embryo does not stain reddish brown is perhaps due to the existence of some carbohydrate of lower class in composition than the dextrin-like substance, which is more diffusible and more available for the use of the growing embryo.

Ovaries in a little later stage than in the above case (11 days after anthesis) show the storage of starch grains in the endosperm tissue. As in the case of dextrin, the greater amount of starch grains a little larger than those in the peripheral region cluster in the middle part and also they make compact oval groups more frequently than in the upper part. These grains are of various polygonal shapes, and their peripheries stain deeply leaving their interiors brilliant. In the lower part, the number of grains is not so abundant as in the middle part, and in the former, one notices very small starch granules in a few layers of endosperm tissue along the back of the scutellum. In the peripheral endosperm region, exclusive of the outermost layer (the aleurone layer) which contains almost none, the grains do not so frequently gather in groups, which is true especially in the outer cells in which starch grains scatter around the nuclei. The distinct differentiation of the aleurone layer from the peripheral outermost cell layer of endosperm tissue completes morphologically, though not physiologically, about at this stage. That is the cells of the aleurone layer have grown twice as large as the peripheral starch endosperm cells (fig. 44).

### **Antipodals**

As above mentioned, the nuclear and cellular division as well as cell growth of antipodals take place at about the time when the contact of

polar nuclei occurs in the central region of the embryo sac.

As far as cytoplasm fixed in FLEMMING's solution comes into consideration, the network of cytoplasm becomes more distinct as the cells grow larger.

Counting the number of antipodal cells is hard, for some are so large as to make a continuous appearance in a few sections cut about  $8\ \mu$  thick. In addition to their irregular arrangement or lobed shape, their boundaries are not very distinct.

Antipodals at about the time of anthesis often exhibit large nuclei with one or several nucleoli, or a few nuclei gathering in a group or a large nucleus produced by the fusion of a few nuclei (fig. 38), and sometimes the cell itself comes to have many vacuoles.

I have never observed mitotic figure of nucleus in these antipodals.

The antipodals which have deeply entered into degeneration, in general make their appearance after the division of the primary endosperm nucleus, and minute granules derived from the degenerated chromatic substance appear in the cytoplasm, deeply staining with haematoxylin. Frequently the large nucleus in the cytoplasm with minute granules as in the above case stains so deeply and homogeneously as to make it hard to identify its nucleoli, and sometimes the entire cell stains so deeply that it is difficult to recognize its nucleoli. The antipodals which represent the further later stage of degeneration the author can see in the ovaries seven days after anthesis (in this case they are fixed in formalin acetic alcohol). They reveal enormously large vacuoles, lacking any minute granules at all which appeared in the above case, and their cell membranes are very distinct (fig. 39).

In general the life of antipodals is pretty long though it depends more on the development of endosperm tissue than the passage of time. In several ovaries fixed in FLEMMING's solution about 56 hours after anthesis, antipodals which have no distinct vacuoles are enclosed within the layer of endosperm nuclei, and even in the ovary which has neither embryo nor endosperm, eleven days after anthesis antipodals still survive.

### The Development of the Embryo

The first division of the fertilized egg cell occurs by a wall transverse to the longitudinal axis of it. The basal and distal cells have no distinguishable difference in size. This two-celled embryo is about  $44\ \mu$  long (fig. 20). P. TANNERT on *Avena sativa* reports "Durch den zweiten Tei-

lungsschritt wird die basale Zelle in zwei übereinanderliegende Zellen geteilt", and PERCIVAL on wheat writes "the large basal suspensor cell apparently undergoing little or no further division.....The upper cell divides by a wall parallel to the first". I can not tell which of these two processes really takes place in *Oryza sativa*; at any rate, there exists a three-celled embryo, the wall of the second division being parallel or oblique to the first (fig. 21). The third division seems to occur in the uppermost cell by a wall perpendicular to the second (fig. 21).

According to my observations in 1925 and 1926, the embryos reach the length of  $68 \mu$  and 0.18 mm. respectively in about 2 and 4 days (in 1925), and  $58 \mu$  and 0.25 mm. respectively in about 3 and 8 days (in 1926) after anthesis. In the upper part of the embryo in this latter stage, a lateral notch appears, which is the first sign of its differentiation (fig. 42). As shown in fig. 22, the younger embryo is a club-shaped mass of cells having its narrow base on the micropylar end of the embryo sac. The embryo eleven days after anthesis is about 8.8 mm. long and 3.6 mm. broad. In the upper part of it, the coleoptile within which plumule is formed, in the lower part, the radicle, root cap, and coleorhiza, and in the part which is in contact with endosperm tissue, the scutellum, have differentiated. At this stage the embryo lies on the ventral side at the base of the grain, so as to be covered by testa on one side as the consequence of displacing the endosperm, with which at first it was completely covered as is usually the case in Poaceae (fig. 43).

The embryo in the later milk-ripened grain about twenty days after anthesis is about 19.4 mm. long and 8.4 mm. broad, and the differentiation of every organ as seen in those eleven days after anthesis, is distinctly found. Transverse microscopical sections in different positions of them show the regular arrangement of the vascular bundles (fig. 46).

### Summary

1. The development of the embryo sac in *Oryza sativa* takes place in an ordinary way as in other forms of Poaceae.
2. The nuclear and cellular divisions of antipodals take place at a very early stage, that is at about the time when the contact of polar nuclei in the central region of the embryo sac occurs.
3. Double fertilization seems to occur in this species.
4. Two nucleoli often appear in the fertilized egg nucleus.
5. In normal conditions, the fertilization takes place about twelve

hours after anthesis.

6. From the first appearance of nucellar tissue up to the development of two or three layers of endosperm, the ovule gradually becomes anatropous.

7. The development of two integuments on the side towards the raphe is not so good as the inner integument on the side opposite it. The outer integument also on the opposite side is very poor in its growth.

8. The outer integument and the outer layer of the inner one disappear respectively in a few days and about 8 days after anthesis.

9. The primary endosperm nucleus completes several successive divisions by the time the fertilized egg cell has reached the metaphase of its first division.

10. In this species there seems to exist the autonomy of both male and female nuclear elements in the fertilized egg and endosperm nuclei.

11. The endosperm formation proceeds with free nuclear division along the periphery of the embryo sac and around the embryo, both in a single layer at first. Then it advances centripetally.

12. In two or three layered tissue of endosperm, the completion of wall formation has been observed.

13. The cells of endosperm tissue are smaller in the periphery and larger inwards.

14. The storage of carbohydrates in the endosperm tissue begins when it has almost completely filled the cavity of the embryo sac. A dextrin-like substance appears in the tissue before starch grains are formed, and both of these substances are stored at first more abundantly in the middle part than in the two terminal parts. Starch grains are more compact in the central region than in the peripheral region.

15. About one day after anthesis a two- to five-celled embryo is seen.

16. The embryo in the later stages lies on the ventral side at the base of the grain so as to be covered by testa on one side as the consequence of displacing the endosperm.

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This work was carried out under the direction of Prof. M. AKEMINE, and I wish to express my hearty thanks to him for his kind advice and suggestions during the long period of my investigation. I am also under obligation to Prof. T. SAKAMURA and Mr. K. HIRATA for their valuable suggestions and help.

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### Explanation of Plates

All drawings were made with the aid of a camera lucida.

#### Plates I and II

Figs. 1-11 and 20-22..... × 385      Figs. 12 and 14-16..... × 920

Figs. 13 and 17-19..... × 660      Figs. 23 and 25..... × 312

Fig. 24..... × 103      Figs. 26-29..... × 51

Figs. 1-12. The process up to the development of the embryo sac from megaspore mother-cell.

Fig. 1. Longitudinal section of ovule; inner and outer integuments, and megaspore mother-cell.

Fig. 2. Dyad of megaspores.

Fig. 3. Triad row of megaspores; the outermost cell is degenerating.

Fig. 4. Tetrad of megaspores; outer two cells are degenerating.

Fig. 5. The innermost cell which begins to enlarge and the debris of three outer cells.

Fig. 6. Two nucleated embryo sac.

Fig. 7. Four nucleated embryo sac.

Fig. 8. Four cells in an antipodal end of embryo sac.

Fig. 9. Three cells in a micropylar end of embryo sac and a polar nucleus starting to migrate.

Fig. 10. Egg cell and synergids, polar nuclei and antipodals, in embryo sac.

Fig. 11. Embryo sac four hours after anthesis.

Fig. 12. Polar nuclei, one of which has two nucleoli.

Fig. 13. The micropylar portion of the embryosac, showing the egg-apparatus and pollen tube by the side of a synergid.

Fig. 14. Male nucleus in the egg cell.

Fig. 15. Early triple fusion.

Fig. 16. Polar nuclei before the triple fusion.

Fig. 17. Egg nucleus with two nucleoli.

Fig. 18. Fertilized egg cell, its nucleus in rest.

Fig. 19. Metaphase of the first division of fertilized egg nucleus with an endosperm nucleus near by.

Figs. 20-22. Longitudinal sections of embryos in different stages of development.

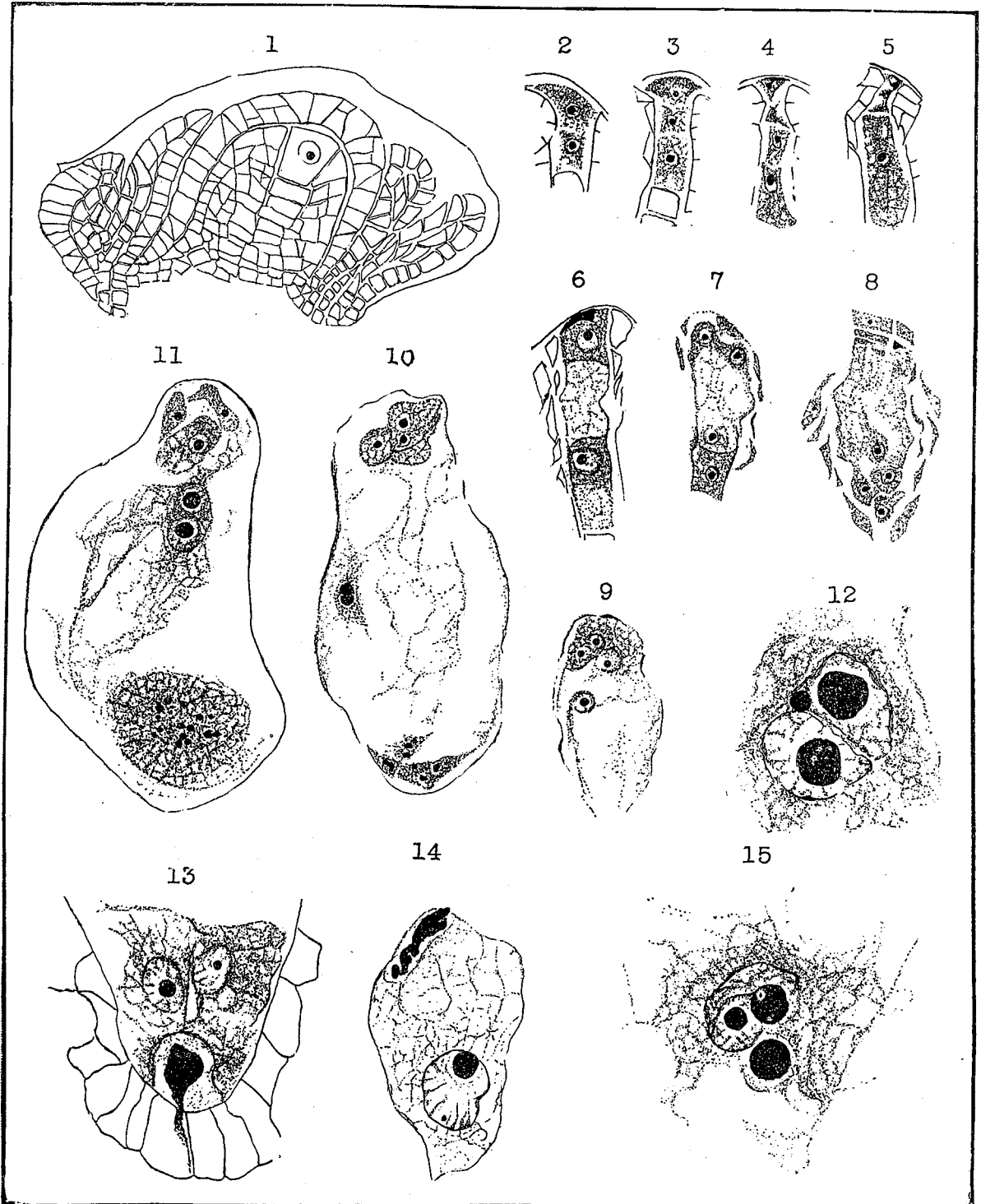
Figs. 20 and 21. Embryos one day after anthesis.

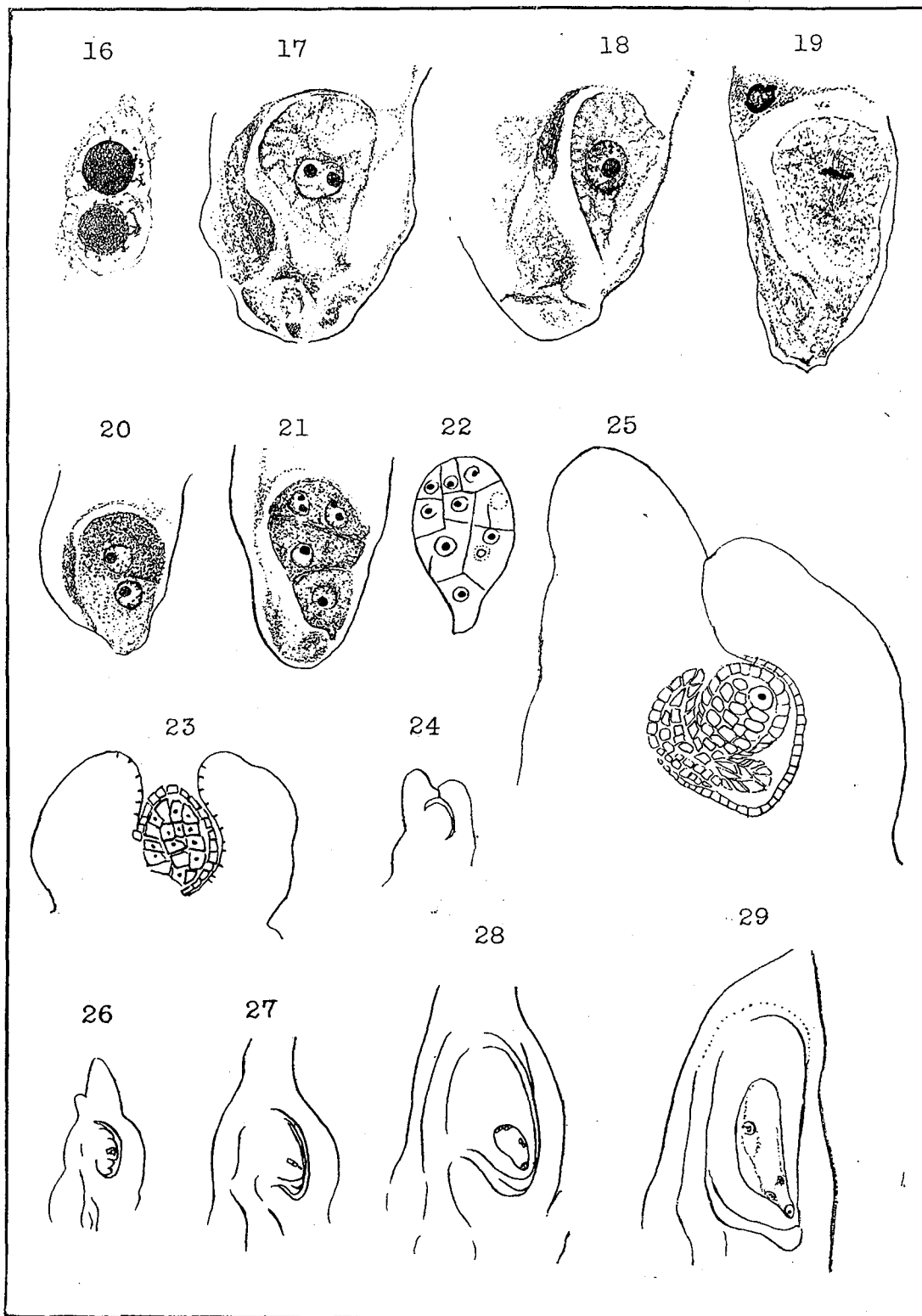
- Fig. 22. Embryo three days after anthesis.  
 Figs. 23-29. Longitudinal sections of ovaries in different stages showing the development and the inclination of the ovule.  
 Fig. 23. The first appearance of ovular tissue.  
 Fig. 24. A later stage of the same.  
 Fig. 25. The first appearance of megaspore mother-cell and inner integument.  
 Fig. 26. Ovary with a matured megaspore mother-cell (the same section as fig. 1).  
 Fig. 27. Ovary with the innermost megaspore and the debris of three outer cells (the same section as fig. 5).  
 Fig. 28. Ovary with egg apparatus, polar nuclei and antipodals in its embryo sac (the same section as fig. 10).  
 Fig. 29. Ovary in the very early stage of endosperm development.

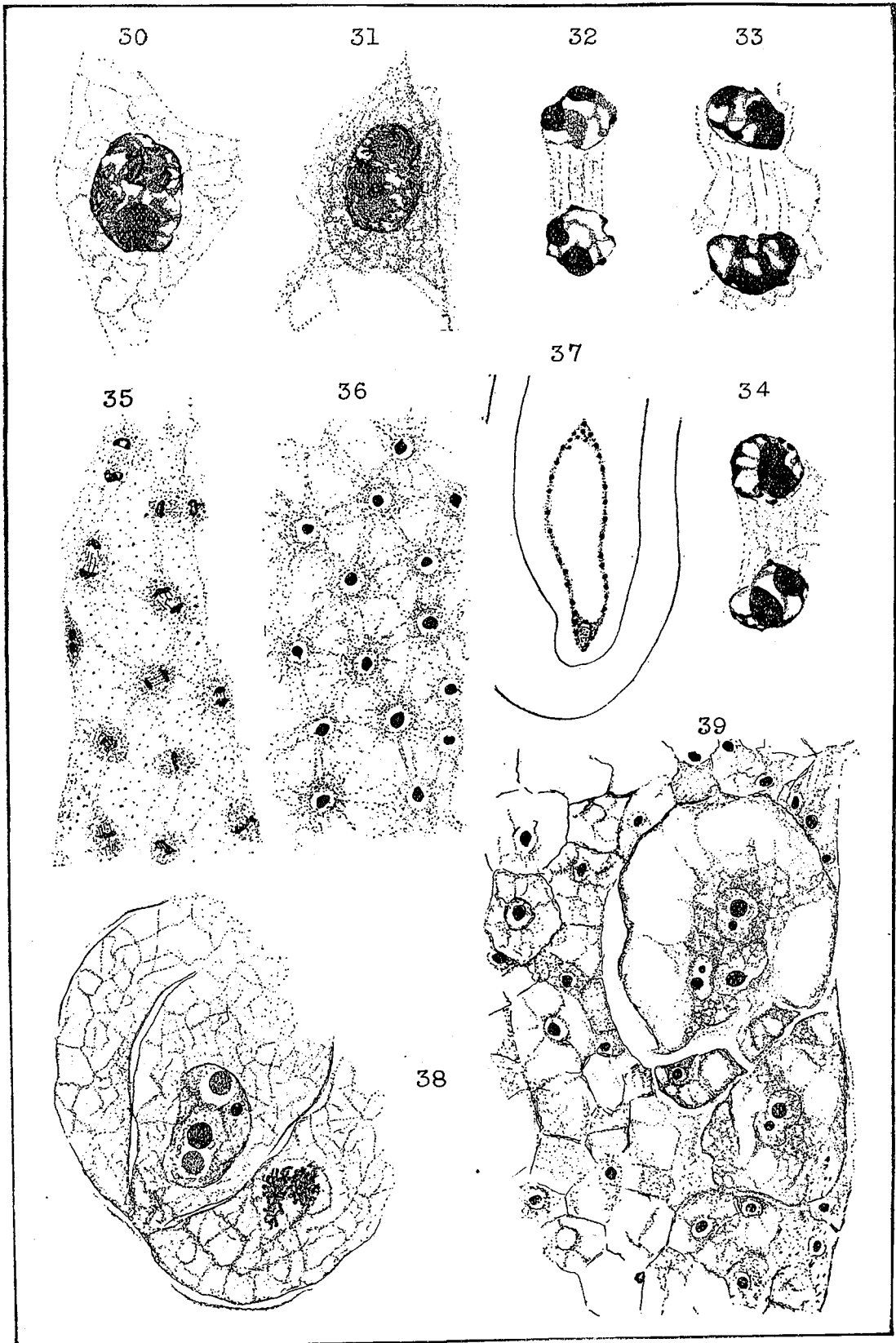
Plates III and IV

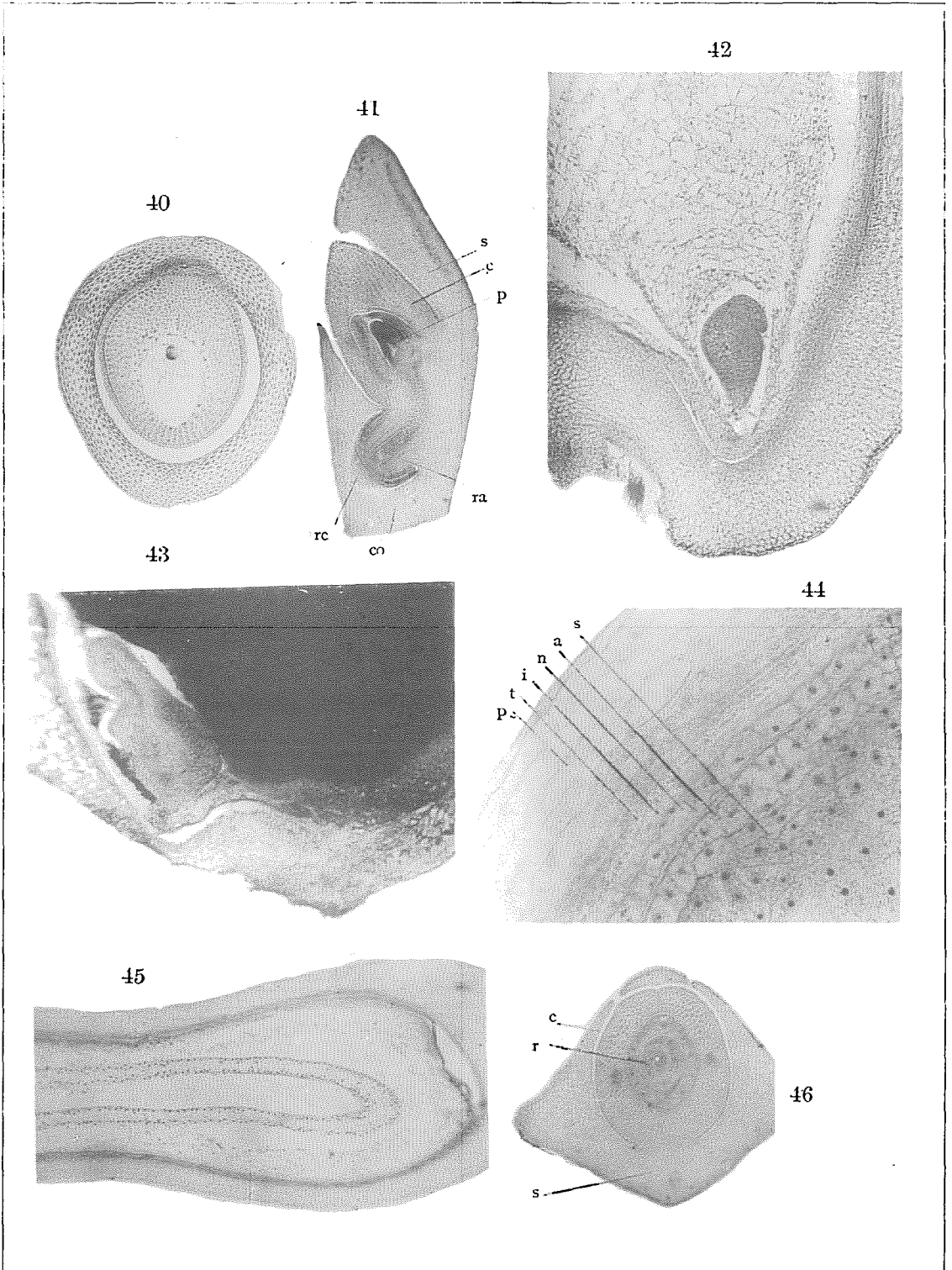
- |                                   |                                   |                   |        |
|-----------------------------------|-----------------------------------|-------------------|--------|
| Figs. 30 and 31.....              | × 920                             | Figs. 32-34 ..... | × 2340 |
| Figs. 35, 36, and 39 .....        | × 385                             | Fig. 37.....      | × 51   |
| Fig. 38.....                      | × 1050                            |                   |        |
| Figs. 40, and 42.....             | Leitz. objective; 3 ocular; 4     |                   |        |
| Figs. 41, 43, 45, and 46.....     | Leitz. objective; 3 ocular; 1     |                   |        |
| Fig. 44.....                      | Leitz. objective; 7 ocular; 1     |                   |        |
| Figs. 39, 41, 44, 45, and 46..... | fixed in formaline acetic alcohol |                   |        |
- Figs. 30-37. Endosperm nuclei.
- Fig. 30 and 31. The chromosomal autonomy in the nuclei derived from the primary endosperm nucleus.  
 Fig. 32 and 33. A few lumps of sticky chromatin in each daughter nucleus.  
 Fig. 34. The upper daughter nucleus shows a large nucleolus probably formed by the fusion of three nucleoli, and the lower, two nucleoli, one of which may be produced by the fusion of two nucleoli.  
 Fig. 35. Surface view of cytoplasmic layer in which endosperm nuclei in their mitosis and homogeneously scattered protoplasmic granules are imbedded.  
 Fig. 36. Radial cytoplasmic threads around endosperm nuclei.  
 Fig. 37. Layer of endosperm nuclei around embryo and embryo sac.  
 Figs. 38 and 39. Antipodals.  
 Fig. 38. Antipodals at the time of anthesis, showing one nucleus produced

- by the fusion of a few nuclei and another degenerating.
- Fig. 39. Antipodals with large vacuoles, within the endosperm tissue seven days after anthesis.
- Fig. 40. Cross section of the middle part of the ovary at about the time of heading showing the meager development of the outer integument and an antipodal cell at the dorsal side of the embryo sac.
- Fig. 41. Embryo sixteen days after anthesis; s, scutellum c, coleoptile; p, plumule; r, rudimentary leaves; ra, radicle; rc, root cap; co, coleorhiza.
- Fig. 42. Embryo in which the first sign of differentiation has appeared; endosperm around the embryo is obliterated, eight days after anthesis.
- Fig. 43. Embryo which lies on the ventral side at the base of grain and endosperm cells which have been already packed with starch grains. Treated with iodine potassium-iodide solution.
- Fig. 44. Transverse section of ovary eleven days after anthesis, p, parenchyma of pericarp; t, tube cells; i, inner layer of inner integument; n, epidermis of nucellus; a, aleurone layer; s, starch endosperm tissue.
- Fig. 45. Two layers of endosperm cells seven days after anthesis in 1926.
- Fig. 46. Transverse section of embryo twenty days after anthesis, showing vascular bundles in the scutellum, rudimentary leaves and coleoptile.
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S. Terada photo.