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A CONTRIBUTION TO THE EMBRYOLOGY OF THE GENUS *PORTULACA*

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INTRODUCTION

MOST embryological investigations on the flowering plants during the past have been concerned with the development of the gametophytes, particularly the embryo-sac. The study of embryonal development has received comparatively little attention. Thus although the development of the embryo-sac and pollen is known in several genera of the Portulacaceæ,—*Calandrinia*, *Claytonia*, *Portulaca*, *Talimum*, *Montia* and *Anacampseros* (Schnarf, 1931)—, the details of embryo development till recently had not been worked out even in one species. Only when the present investigation had been nearly completed, there has appeared a paper by Souèges (1938) describing the embryo development in *Portulaca oleracea*.

During the course of the investigation the writer has studied the development of endosperm and embryo in *Portulaca grandiflora* Hook., *P. oleracea* Linn. and *P. quadrifida* Linn. In addition to this the development of pollen and embryo-sac in *Portulaca quadrifida*, a species which has hitherto received no attention, is also described.

The material of all the species was collected from plants growing in the Botanical Garden of the Benares Hindu University. *Portulaca grandiflora* is a common garden plant, while *P. oleracea* and *P. quadrifida* are common weeds throughout the country. Fixations were made in the morning between 7 to 10 A.M. during the winter season. This time is quite suitable for getting the mitotic divisions in the proembryo. Three different fixatives were used, namely, Nawaschin's fluid, Formalin-acetic-alcohol and Carnoy's fluid. The first two fixatives proved quite satisfactory. Depending upon the stage of development, sections were cut at a thickness ranging from 8 to 14μ. For the study of embryo development ovules were dissected out of

the gynœcium and sectioned individually. Delafield's Hæmatoxylin, Heidenhain's Iron-alum Hæmatoxylin, Ehrlich's Hæmatoxylin and a combination of Safranin and Gentian violet were used as stains. The last combination is more satisfactory than others for the study of seed-coat development, particularly during the early stages, the grains deposited in the layers of the testa looking bright violet. As recommended by Cooper (1935), Ehrlich's Hæmatoxylin was used for the study of pollen grains, which are rich in starch grains. But it was not found so satisfactory. In order to remove the starch grains, slides were treated with aqueous solution of Taka Diastase and then stained. This slightly improved the staining. The external characters of the pollen grains were studied in Methyl-green glycerine-jelly mounts.

Microsporogenesis

The development of the anther and pollen in *Portulaca grandiflora* has been worked out by Dahlgren (1916), Rócen (1927) and Tjebbes (1928). Rócen (1927) has also investigated *Portulaca oleracea*. He found that it does not differ much from *Portulaca grandiflora* in the development of floral structures. The development of the male gametophyte in the former species has been further described by Cooper (1935). This aspect of the life-history, therefore, has been studied by the present author only in *Portulaca quadrifida*.

The anther is four-lobed. The primary archesporium in each lobe is confined to a single hypodermal row of four to six cells (Fig. 1). Each archesporial cell cuts off a parietal cell towards the outside and the primary sporogenous cell towards the inside (Fig. 1). The primary sporogenous cells directly function as the pollen-mother cells, as described by Cooper (1935) in *Portulaca oleracea* (Figs. 2 and 3). From the observations of Rócen (1927) on other genera it appears that this feature is characteristic of the Portulacaceæ. The pollen-mother cells even from the beginning are much bigger than the surrounding cells (Fig. 1). An unusual feature is the frequent occurrence of vacuoles in the protoplasm of the pollen-mother cells (Figs. 2 and 3). The cells of the primary parietal layer divide anticlinally and periclinally to form two layers (Fig. 2). One adjacent to the spore-mother cells divides once more periclinally (Fig. 3). In this way the primary parietal layer forms three layers. Out of these three layers, the middle one is crushed early during further development. The layer in the hypodermal position develops into the fibrous endothecium. The innermost parietal layer forms the tapetum. During the reduction division of the pollen-mother cells the single nucleus of the tapetal cells divides once mitotically and the cells thus become two-nucleate. In the material examined, they were neither observed to become multi-nucleate, nor their nuclei were seen to divide amitotically, as has been reported by Rócen (1927) in *Portulaca grandiflora*. The tapetal cells during their entire life were never seen to leave their place to form any periplasmodium. They thus appear to be purely secretory in function. Granular cutinisation of the inner surface of the

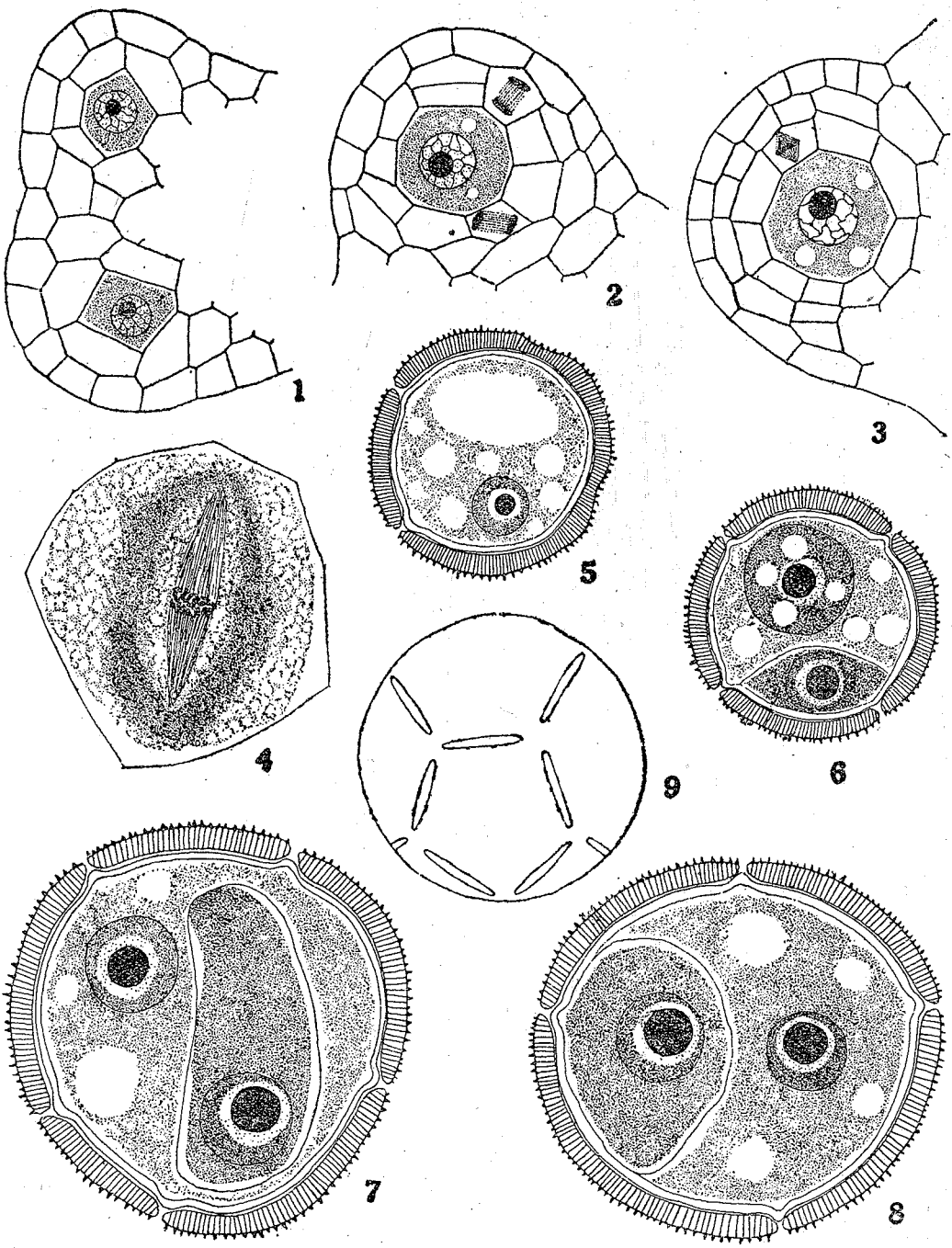
tapetal and the fibrous endothecial cells has been observed as in several other Centrospermales (Kajale, 1940 *b*).

During the first meiotic division the spindle fibres in the pollen-mother cells are surrounded by a dense zone of cytoplasm as reported by Cooper (1935) in *Portulaca oleracea* (Fig. 4). At the same time each pollen-mother cell is surrounded by a distinct sheath of mucilage, which appears between the protoplasm and its original wall (Fig. 4). It persists till the pollen-mother cell divides to form the tetrad. After the disintegration of this sheath and the original cell wall the four pollen grains separate off from one another. A large percentage of them are arranged in a tetrahedral manner, while intermixed with the latter a few are seen to form isobilateral tetrads.

The young pollen grain is rich in protoplasm. Very soon it develops the exine and intine, and at the same time it begins to increase in size. As a consequence of this enlargement it becomes vacuolate (Fig. 5). The nucleus along with some cytoplasm is pushed to the periphery. Here it divides mitotically into two nuclei, which are separated from each other by a curved wall. Thus the generative cell is organised (Fig. 6). It is interesting to note that in a few instances small vacuoles were observed in the tube nucleus (Fig. 6). The behaviour of the generative cell in *Portulaca quadrifida* is seen to differ from the angiosperms in general. In the flowering plants generally this cell is ephemeral, the new wall disappearing almost as soon as it is formed. It is, therefore, not possible for the cell to grow in size or change its shape. In the present material, however, the generative cell increases in size along with the increase in the dimensions of the pollen grain (compare Figs. 6 to 8). Its form varies with the plane in which it is cut (Figs. 7 and 8). It also stains slightly deeper than the other parts. The generative cell, however, does not persist till the shedding time of the pollen grain. Before this stage is reached, the cell wall of the generative cell disappears. The nucleus divides. This is followed by the division of its cytoplasm and two somewhat elongated male cells with a distinct sheath of cytoplasm surrounding the nucleus are formed. Male cells have also been reported in *Portulaca oleracea* by Cooper (1935) and have been seen by the writer in *Portulaca grandiflora*.

Each pollen grain as usual has intine and exine, the former lining the latter all round from inside. The thickness of the intine differs in the three species. It is thicker in *Portulaca oleracea* than in the other two species and is slightly thinner than the exine.

The exine is perforated generally by 30 furrows so arranged as to form twelve exactly similar pentagons (Fig. 9). Fischer (1890) has described a similar figure of the pollen grains of *Portulaca oleracea* and *P. grandiflora*. Franz (1908) has also pointed out that the dodecahedron is the usual configuration for the microspores in a large number of plants of this family. The length of the germinal furrows varies in the different species. They are comparatively narrower and longer in *P. quadrifida* than in the other two species.



Figs. 1–9. *Portulaca quadrifida.* Figs. 1–3. Transverse sections of anther-lobes at various stages of development. In Figs. 2 and 3 vacuoles

In *P. grandiflora* they are quite broad and the length is only about 2–3 times the maximum breadth. They are thus roughly ellipsoidal in outline. The germinal furrows do not possess any germ pore and themselves function as the passage for the outgoing pollen tube.

The structure of the exine is just the same as described by the author for members of the Amarantaceæ (Kajale, 1940 *b*). As seen in section, the exine is found to be composed of alternating light and dark staining portions, the latter appearing rod-like (Figs. 5 to 8). During the later development of the pollen grain the exine gets thinner than before. The cause of this change is discussed by the author elsewhere (Kajale, 1940 *b*).

The structure of exine is further interesting in that it bears numerous spines on its outside. The spines in different species differ in their length. In general they are bigger in *P. grandiflora* than in the other species. The smallest spines are observed in *P. quadrifida*, while *P. oleracea* is intermediate between the two species so far as this character is concerned. In *P. quadrifida* the spines are so small that they may be easily overlooked.

The pollen grains are shed at the three-nucleate stage as in the Centrospermales in general. Their diameter is 75 to 80 μ in *Portulaca grandiflora*, 73 to 77 μ in *P. oleracea* and 66 to 70 μ in *P. quadrifida*.

Structure of the Ovary and Ovule

The ovary is semi-inferior in the genus. It is generally trilocular (Figs. 10 and 12), but sometimes more loculi may be seen. It develops from the tip of the floral axis after the other parts have been differentiated. Along with the development of the ovary wall septa equal to the number of the loculi begin to develop from the wall. The ovules develop from the septa as lateral outgrowths (Fig. 10). The septa meet in the centre of the gynœcium resulting in the axile placentation (Fig. 10). The fusion of the septa takes place throughout the length of the gynœcium, unlike in *Sesuvium Portulacastrum* (Kajale, 1940 *a*), where the septa do not meet in the upper part. The entire central axis, however, does not bear ovules. The latter are confined mostly to lower half of the placentas, while the upper half is barren (Fig. 11). Further the upper part does not persist during the development of the fruit (Figs. 11 and 13). Consequently the axile placentation becomes almost free central during the later stages (Fig. 11).

The development of the ovule has been studied in *Portulaca quadrifida*. The first indication of the ovule is seen in the form of

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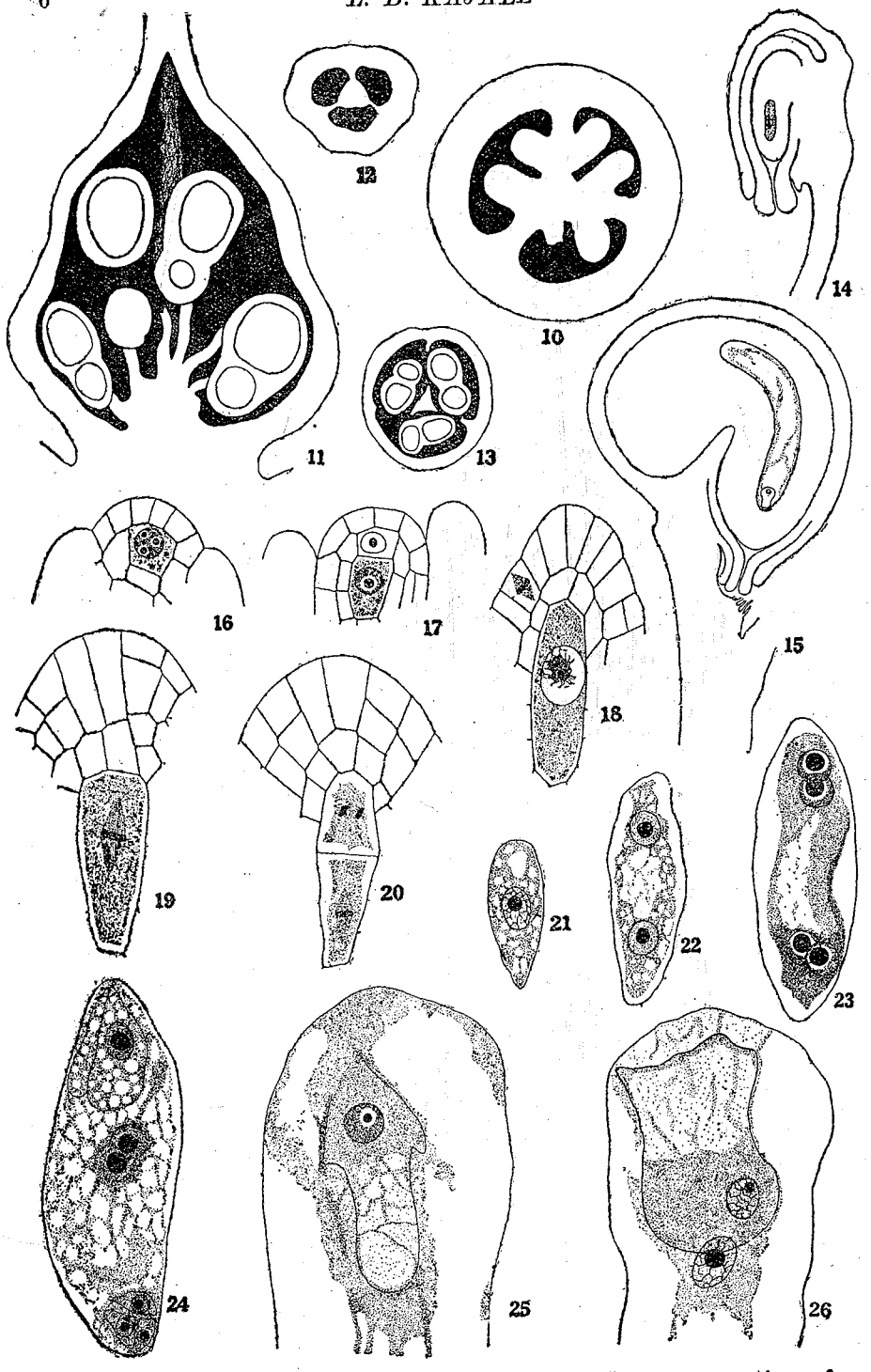
are seen in the cytoplasm of the pollen-mother cells. Fig. 4. A pollen-mother cell in the metaphase of the I meiotic division; a perinuclear zone is seen round the spindle. Fig. 5. 1-nucleate pollen grain. Figs. 6–8. 2-nucleate pollen grains. Generative cell increases in size and presents different shape when cut in different planes. Fig. 9. A pollen grain in surface view showing the arrangement of the furrows. (Figs. 1–3 and 5–8 × 450; Fig. 4 × 800; Fig. 9 × 250.)



Figs. 10–26. *Portulaca quadrifida*. Fig. 10. Transverse section of a ovary showing the developing ovules and their arrangement. Fig. 11. Longitudinal section of the ovary with ovules at the mature embryo-sac

a small protuberance on the margins of the ingrowing septa (Fig. 10). It soon becomes bent in the upper part due to pronounced unilateral growth. The archesporium appears about this stage and the two parts, namely, the comparatively narrow funicle and the ovule proper become distinct. The funicle in the mature ovule is fairly long and erect (Fig. 15). Each ovule possesses two integuments which appear as annular outgrowths from the base of the nucellus. The differentiation of the integuments in the ovule is almost synchronous with the development of the archesporium (Fig. 16). By the time the megaspore-mother cell is well differentiated the ovule has developed to a sufficient extent to assume nearly an anatropous form (Fig. 14). The integuments by this time have covered almost the entire nucellus. In between them at the chalazal end a prominent air-space is observed (Fig. 14), a feature which appears to be characteristic of the whole order Centrospermales. Both the integuments do not take part in the formation of the micropyle. As in the Centrospermales in general, the inner integument grows ahead of the outer and alone forms the micropyle (Fig. 15). The integuments are mostly two cells thick, except at the micropylar extremity, where they are thicker.

The nucellus about the megaspore-mother cell stage is two layers thick at the apex, three to five layers thick at the sides, and nine to ten layers thick below the megaspore-mother cell. Along with the development of the female gametophyte, the nucellus also develops further, particularly in the chalazal region. The epidermal cap over the nucellus is formed in a characteristic manner. Those cells situated just below the micropyle merely stretch out radially, while the surrounding cells divide by periclinal walls (Figs. 18 to 20). This corresponds to what has been observed in several genera of the Ficoidaceæ, namely, *Mesembrianthemum* (Schmid, 1925), *Trianthema* (Bhargava, 1935) and *Sesuvium* (Kajale, 1940 *a*).

MEGASPOROGENESIS

Simultaneously with the development of the integumental initials one cell of the nucellus just below the epidermis begins to take a deeper stain than the rest, increases in size and differentiates

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stage. Dotted part represents the part of the central axis that disappears during further development. Figs. 12–13. Transverse sections of the ovary at the apex and base respectively about the time of fertilisation. Figs. 14–15. Ovules at 1-nucleate and mature embryo-sac stages. Fig. 16. A young nucellus showing a single archesporial cell. Fig. 17. Another nucellus showing a megaspore-mother cell. Fig. 18. A megaspore-mother cell during the prophase of the I meiotic division. Fig. 19. A megaspore-mother cell in the I metaphase. Excluding the central cells other cells of the nucellar epidermis have divided periclinally to form an epidermal cap. This is also shown in next figure. Fig. 20. Dyad dividing. Figs. 21–23. Various stages in the development of the embryo-sac. Fig. 24. 7-Nucleate embryo-sac showing two synergids, secondary nucleus and three antipodals. Fig. 25. Micropylar part of a mature embryo-sac showing one of the synergids. Fig. 26. The same showing the egg and one polar nucleus. (Fig. 10 × 100; Figs. 11–13 × 25; Fig. 14 × 100; Fig. 15 × 75; Figs. 16–26 × 450.)

as the primary archesporial cell (Fig. 16). It cuts off a parietal cell (Fig. 17). The resulting megaspore-mother cell increases in volume until it has reached the specific maximum size (Fig. 18). Now its nucleus prepares for the meiotic divisions. These are quite normal. After the first division a cell wall is formed and two dyad cells are organized (Figs. 19 to 20). The first meiotic spindle is present in the upper part of the megaspore-mother cell (Fig. 19). Hence the micropylar dyad cell is generally smaller than the chalazal one. Both of them undergo the II meiotic division almost simultaneously and a linear row of four megaspores is formed. At times the spindle in the micropylar dyad is arranged at right angles to that of the lower dyad (Fig. 20). Such an arrangement of the spindles leads to the formation of a T-shaped tetrad. Of the four megaspores only the chalazal one develops into the embryo-sac. The other three towards the micropyle degenerate very rapidly.

The uni-nucleate embryo-sac may be very much vacuolate (Fig. 21), or may possess a few vacuoles on either side of the nucleus. Its nucleus divides mitotically into two. The daughter nuclei travel towards the two poles of the embryo-sac and on reaching there divide once again to form a four-nucleate embryo-sac (Figs. 22 and 23). These four nuclei divide once again mitotically and a typical eight-nucleate embryo-sac is formed.

The egg-apparatus is organized in the normal manner. The egg is a broad flask-shaped structure. Its micropylar end is vacuolate. The nucleus with cytoplasm is present in the basal region (Fig. 26). Some difference in the size of the egg in different plants has been frequently observed (Figs. 26 and 28). The synergids are two in number. The nucleus is present in the upper part from the very beginning (Fig. 24). They have prominent hooks and the nucleus may be situated near the hooks or slightly above them (Figs. 25 and 27). The young synergids are prominently vacuolate, the vacuoles being present all over the synergids (Fig. 24). It appears that as the synergids become mature these vacuoles fuse to form a large basal vacuole (Fig. 25). As in the case of eggs a difference in the size of the synergids in different plants is also observed (compare Figs. 25 and 27). The antipodals are three small cells occupying the chalazal end of the embryo-sac (Fig. 24). Sooner or later they degenerate and no trace of them is seen during the embryo development. Out of the two polar nuclei the lower one moves towards the micropylar end of the embryo-sac, so that both the polar nuclei come to lie closely pressed against each other somewhere in the vicinity of the egg (Figs. 26 and 27). During this time they also show an increase in size and are bigger than any other nuclei in the embryo-sac. Their further fate is described below.

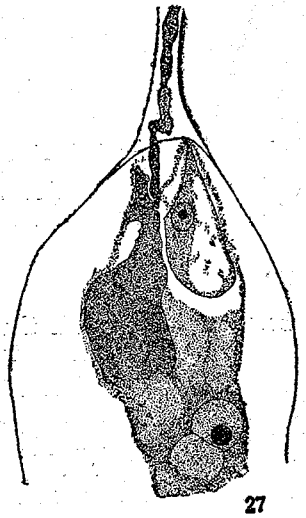
Fertilisation

The details of fertilisation have been studied in *Portulaca quadrifida* only. The pollen grains reach the stigma in the three-nucleate condition. There they begin to germinate rather quickly. The ends of pollen tubes can be clearly seen inside the embryo-sac

of flowers fixed within an hour or two from the time of their opening. Artificial culture of the pollen grains also shows the same behaviour. It has been found that the pollen grains can germinate very easily even in tap water. When they are placed in a drop of water, they burst almost immediately and the pollen tubes come out in no time.

A large number of pollen grains germinate on the stigma and hence many pollen tubes can be observed on their way to the ovules inside the style. Further down they make their way through one of the placentas until they reach an ovule. Now they pass through the micropyle into the nucellus and penetrating it they break through the apex of the embryo-sac.

The end of the pollen tube on entering the embryo-sac swells up enormously, so much so that it fills the greater part of the micro-



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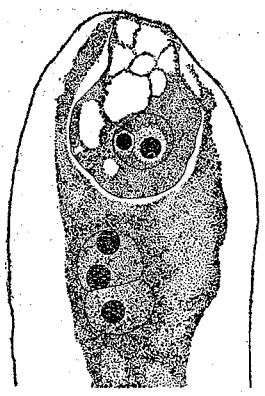

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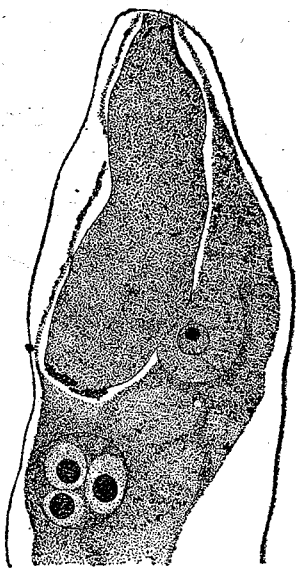

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Figs. 27–29. *Portulaca quadrifida.* Fig. 27. Micropylar part of an embryo-sac showing one synergid, remains of the pollen tube and two polar nuclei. Fig. 28. The same showing the fusion of the male gametes with the egg nucleus and the polar nuclei. Fig. 29. The same showing the remains of the pollen tube, egg and triple fusion (× 450).

pylar end of the embryo-sac (Figs. 27 and 29). On its way through the embryo-sac the pollen tube passes along one of the synergids, which as a result is destroyed completely, while the other synergid, as in *Salix* (Chamberlain, 1897), *Silphium* (Merrel, 1900), etc., is left intact (Fig. 27). Some times, however, both the synergids were seen to be destroyed by the entrance of the pollen tube. The extreme tip of the pollen tube extends beyond the lower end of the egg and there its contents are discharged (Figs. 27 and 29).

Out of the two gametes brought by the pollen tube one fuses with the egg. It rounds up and passing inside the egg unites with its nucleus. Just before the fusion is complete a round deeply staining body is seen inside the nucleus of the egg (Fig. 28). Ultimately it fuses with the nucleolus of the egg. Unlike in the large majority of angiosperms, the polar nuclei do not fuse to form a secondary nucleus before fusing with the male gamete. They remain separate till the male gamete reaches them (Fig. 28). The triple fusion then takes place almost simultaneously between the two polar nuclei and the second male gamete (Figs. 28 and 29). The individual boundaries of the three nuclei disappear and three nucleoli are seen enclosed within a common nuclear membrane (Fig. 29). They later fuse to form a big triploid nucleus. The second male gamete also undergoes change in shape like the one fusing with the egg.

The Embryo

This phase of life-history has been studied in all the three species, but *Portulaca quadrifida* has been studied more critically.

The fertilised egg first undergoes a transverse division (Figs. 30, 31 and 61). The two cells formed as a result of this division behave differently during further development. The micropylar cell generally divides before the other (Figs. 32 and 62). It may divide once or twice to form up to three cells before any transverse division takes place in the apical cell (Figs. 32, 63 and 64). At times it may not divide also (Fig. 38). This cell, therefore, varies a good deal in its behaviour. The transverse divisions in the apical cell follow a certain definite sequence. First it divides transversely (Figs. 33 and 64). The resulting apical cell does not divide any more in a transverse plane for some time, while the penultimate cell divides transversely to form two cells as shown in Fig. 34. The one adjacent to the apical cell does not divide again transversely, while the other divides into two cells (Figs. 36, 39 and 65). Next transverse division takes place in the fourth cell from the apex (Fig. 54). By now four cells result from the apical cell of the two-celled proembryo. In this way depending upon the behaviour of the micropylar cell a proembryo of five to six or sometimes seven cells is organized in *P. quadrifida* (Figs. 35 and 36). A similar organization of the proembryo is observed in *P. grandiflora*. This will be clear from Figs. 61 to 65. Some stages were observed in *P. oleracea* also and they leave no doubt that the proembryo in that species also is organized on the same lines. The length of the proembryo in the

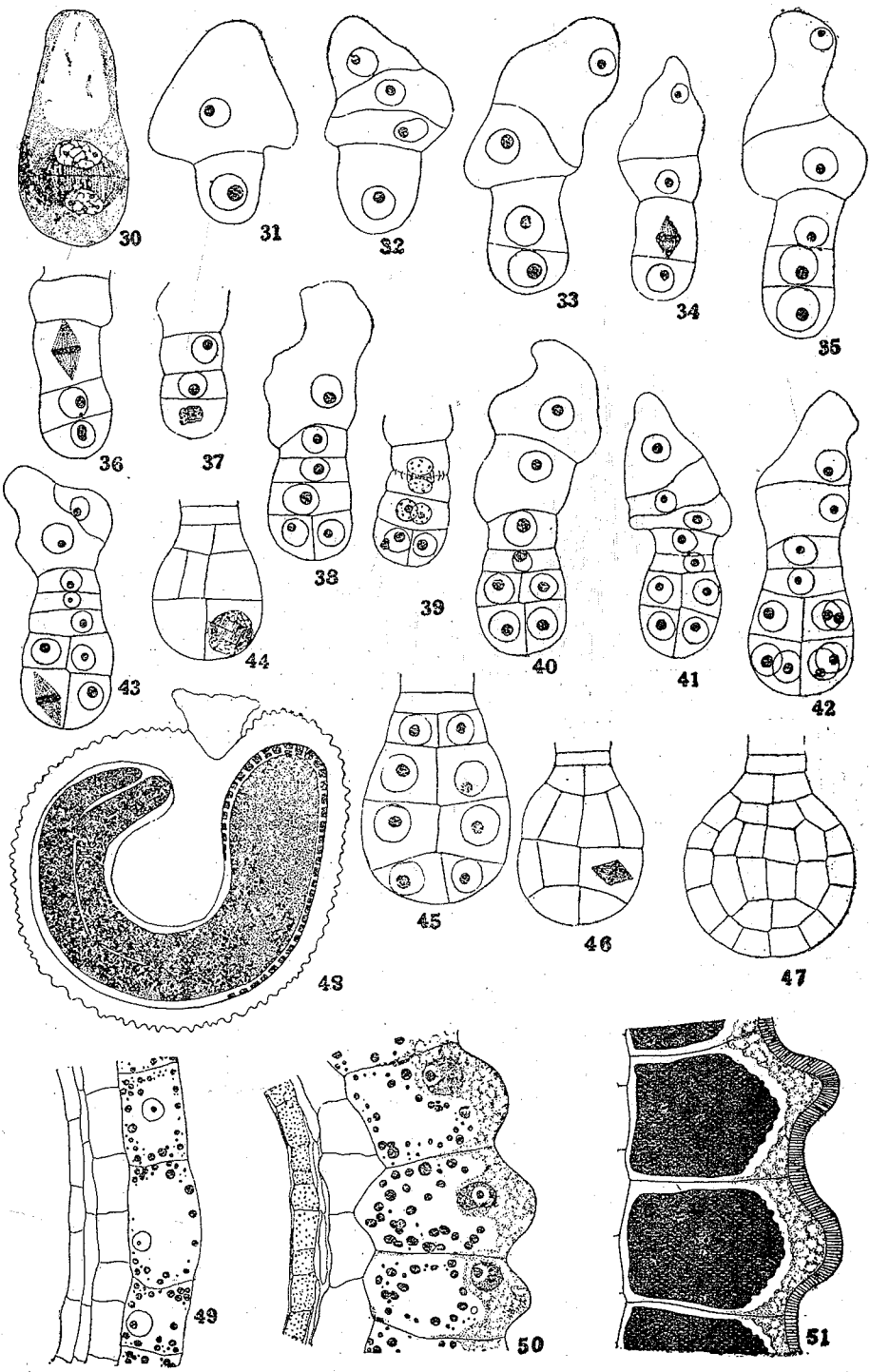
latter two species is also five to six cells as in *P. quadrifida* (Figs. 52 and 66).

Out of five to seven cells of the proembryo the embryo proper develops from the three apical cells of the former. These three cells and one or two more cells adjacent to them come entirely from the apical cell of the two-celled proembryo. The further differentiation of these three cells into different parts of the mature embryo may be stated as follows. The apical cell forms the stem tip and the two cotyledons. The penultimate cell forms the hypocotyl and the greater part of the radicle. The third cell from the apex completes the apex of the radicle and, therefore, is the hypophysis.

This naturally takes us to the details about the further development of these different cells. The apical cell is the first to divide in a longitudinal manner (Figs. 38, 52, 53 and 66). Another longitudinal wall at right angles to the first appears in the same cell and four cells are formed (Figs. 42 and 55). All of them divide now transversely to form an octant (Figs. 43, 44, 57 and 58). In the meanwhile, the penultimate cell also divides longitudinally (Figs. 39 to 43 ; 54 to 58 and 67). The plane of this division generally is at right angles to that of the first longitudinal division in the apical cell. Along with the octant formation in the apical cell the penultimate cell also divides once more and four cells are formed. The sequence about the appearance of longitudinal divisions is, therefore, from the apical towards the micropylar cells. One important fact emerges out of this. Within the Centrospermales a group of families can be set apart, where the first longitudinal division appears in the apical cell and then it progresses towards the micropylar end. Illustrations of this type are found in the Nyctaginaceæ (Kajale, 1938), and the Phytolacaceæ (Kajale, unpublished). Some indications of the occurrence of similar sequence of longitudinal divisions in the Molluginaceæ are seen in the figures of Bhargava (1934, text-figures 5 and 6). In another group of families longitudinal division in the proembryo starts either in the second, third, fourth or even fifth cell from the apex and then it gradually extends towards the apical cell of the proembryo. This sequence is just the reverse of what is found in the first group of families, and its examples are found in the Chenopodiaceæ (Souèges, 1920), Caryophyllaceæ (Souèges, 1924) and Amarantaceæ (Joshi and Kajale, 1937).

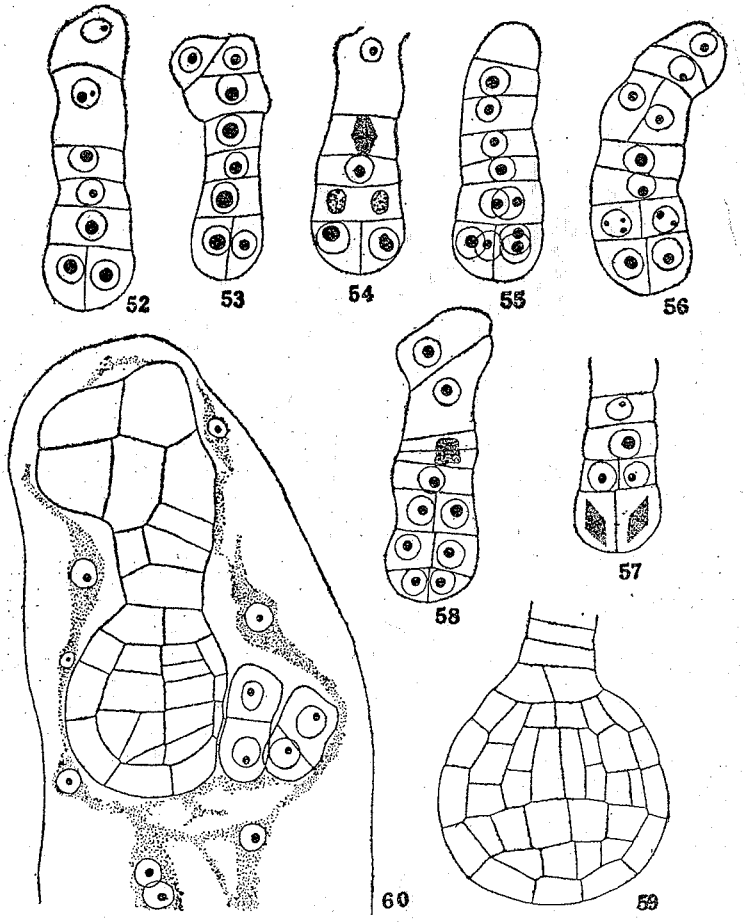
After the longitudinal walls have been completed in the apical and penultimate cells, periclinal divisions begin to appear (Figs. 44 and 68). This is the beginning of the dermatogen differentiation. It begins in the tier formed by the penultimate cell and is later completed in the apical tiers (Figs. 44, 46, 68 to 71). It should be noted here, that the basipetal differentiation of the dermatogen appears distinctly characteristic of the Centrospermales and no deviation from this rule has yet been observed in any member of this order.

After the completion of the dermatogen, the divisions in the tiers formed by the apical and penultimate cells are not equal. The tier resulting from the latter cell divides more actively than the other



Figs. 30–51. *Portulaca quadrifida.* Figs. 30–47. Various stages in the development of embryo. In Figs. 44–47 suspensor is not shown. Fig. 48. A longitudinal section of the mature seed showing the embryo,

both by transverse and longitudinal walls, and forms the hypocotyl and the greater part of the radicle. As seen in the longitudinal section at about the stage when the cotyledons appear (Fig. 73), one or two peripheral layers below the dermatogen function as the periblem, while the central core enclosed by the latter forms the plerome.



Figs. 52–60. *Portulaca oleracea.* Figs. 52–59. Various stages in the development of the embryo. In Fig. 59 suspensor is not shown. Fig. 60. Micropylar part of the embryo-sac showing three embryos developing side by side (× 450).

The cells of the two tiers resulting from the apical cell divide chiefly by longitudinal walls (Figs. 72 and 73). After the embryo has reached the size shown by Fig. 73 the central part develops into the

perisperm and a layer of endosperm. Figs. 49–51. Various stages in the development of testa. (Figs. 30–47 × 450; Fig. 48 × 50; Fig. 49 × 450; Figs. 50–51 × 250.)

stem tip, while the two cotyledons are formed from the peripheral region. Once the cotyledonary initials are formed their growth is very active and two large cotyledons are formed (Figs. 48 and 74).

The third cell from the apex deserves consideration now. This cell functions directly as the hypophysis. It first divides by two longitudinal walls forming four cells simultaneously with the differentiation of dermatogen in the apical tiers (Figs. 45, 46, 58 and 68). These cells do not divide further till rather a late stage of embryo development. Ultimately they complete the apical part of the radicle including the root cap.

Excluding the three apical cells of the proembryo the remaining cells develop into the suspensor. It should be noted that the suspensor does not exclusively develop from the micropylar cell of the two-celled proembryo, but is also formed by a few cells derived from the apical cell. The structure of the suspensor differs in the three species of *Portulaca*. In *P. quadrifida* it consists of three to five cells only. It does not become massive in any part and generally remains uniseriate till it degenerates (Figs. 41 and 43). In the other two species it is more massive particularly at the micropylar end (Figs. 53, 68–70 and 73). Towards the embryo it is mostly uniseriate. This will be clear from Figs. 53 and 68–73. In *P. oleracea* it is observed that the cells of the suspensor sometimes divide by longitudinal walls quite at an early stage (Fig. 53). In all the three species no trace of the suspensor is seen in the mature embryo.

Mature Embryo

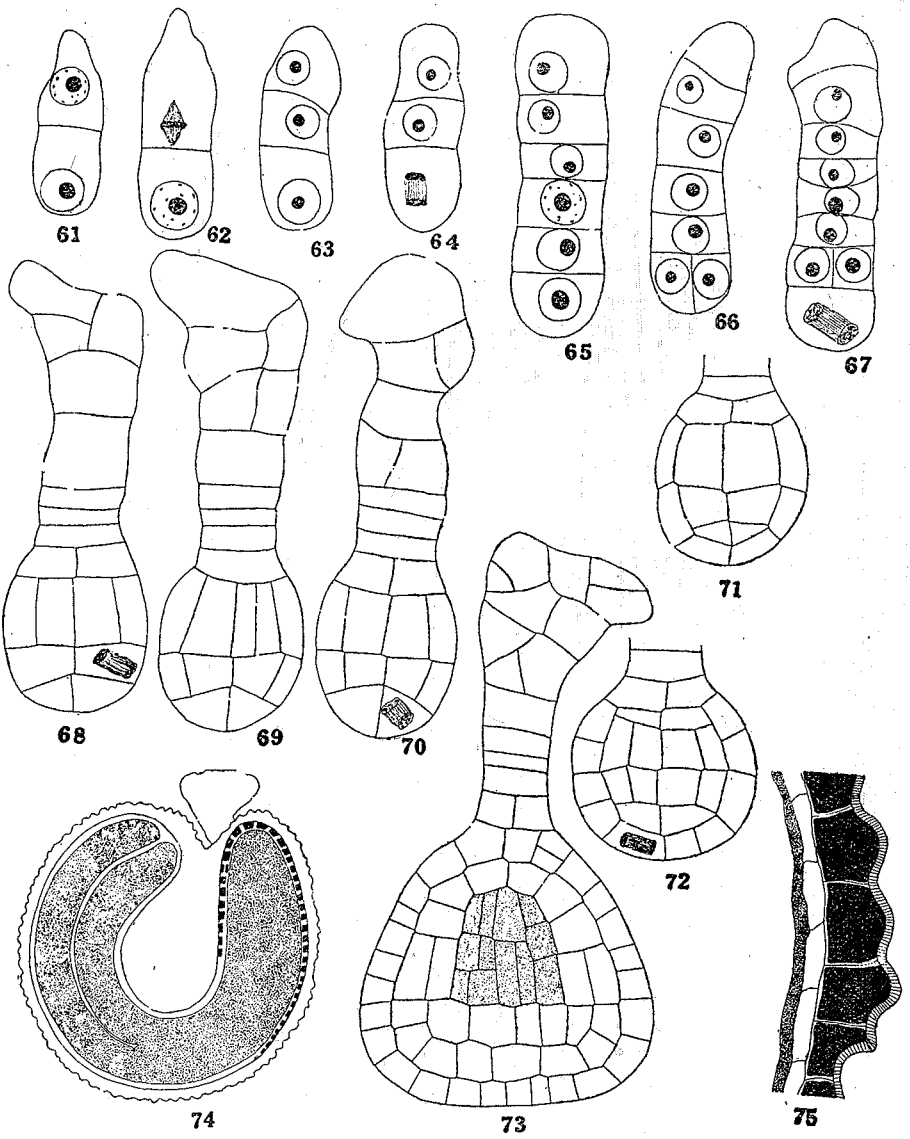
The mature embryo is annular as in the Centrospermales in general (Figs. 48 and 74). It encloses within itself a large part of the nucellus that persists in the mature seed as perisperm. Abundant starch grains are deposited in the embryo. Roughly speaking these are uniformly distributed in the different parts of the embryo, except in the apical part of the radicle, where comparatively less starch is deposited. The size of the starch grains is also smaller in this part. In the region of plerome again the starch grains are smaller in size than in the other histogenic layers.

Polyembryony

A case of polyembryony has been observed in *Portulaca oleracea*. It is sketched in Fig. 60. There are three embryos developing side by side in the same embryo-sac. All these embryos are not developed to the same extent. One of them is much bigger than the other two. In the bigger embryo the dermatogen formation has been completed and the presence of suspensor, which is massive in the micropylar part, is also clearly seen. The other two embryos have not developed so much. Each one of them consists of two cells only. It follows from this that they have either developed much later than the bigger embryo, or their development has been slow.

The bigger embryo appears to have developed in the usual manner from a normal fertilised egg. The origin of the other two embryos is not very clear. Perhaps they have developed from

endosperm nuclei. At a stage represented by the bigger embryo in other embryo-sacs the structures other than the egg and endosperm nuclei, *i.e.*, the synergids and the antipodals, all disappear.



Figs. 61–75. *Portulaca grandiflora*. Figs. 61–73. Various stages in the development of the embryo. In Figs. 71 and 72 suspensor is not shown. Dotted part in Fig. 73 represents the plerome. Fig. 74. Longitudinal section of the mature seed showing embryo, perisperm and a layer of endosperm. Fig. 75. A part of the testa as seen in longitudinal section. (Figs. 61–72 × 450; Figs. 73 and 74 × 400; Fig. 75 × 250.)

It is, therefore, unlikely in the present instance that these embryos could have developed either from the synergids or the antipodals. Likewise they could not have developed from the nucellus cells since they are situated well within the embryo-sac. They are not connected with the cells of the nucellus. Their origin appears to be from two endosperm nuclei. Authentic cases of the development of embryos from endosperm cells are very rare. As a matter of fact previously no example is known where an embryo has actually been seen developing from endosperm that had been produced as a result of triple fusion. Jeffrey and Haertl (1939) found in *Trillium* that embryos are derived from the endosperm nucleus, but in this case neither the egg nor the endosperm nucleus is fertilised. Two geneticists, Yamamoto (1936) and Kostoff (1939), however, have suggested that triploids in wheat and rye respectively develop from the endosperm, since in polyembryonous seeds they occur more frequently than haploids in conjunction with diploids.

Perisperm and Endosperm

The central part of the nucellus persists in the mature seed as perisperm. This is enclosed by the embryo. It is a pear-shaped body with cells full of starch grains.

The endosperm formation begins at first in a free nuclear fashion. Later the endosperm becomes cellular. The wall formation starts at the micropylar end of the embryo-sac as observed by Joshi and Kajale (1937) in *Alternanthera sessilis* and *Digera arvensis*. It then extends towards the chalazal extremity and the endosperm completely becomes cellular. In this respect my observations differ from those of Rócen (1927). He says that in *Portulaca oleracea* the endosperm becomes cellular only in the micropylar part of the embryo-sac. In the slides I have examined the endosperm in all the three species of *Portulaca* becomes completely cellular during the development of the embryo. The greater part of it is absorbed by the growing embryo and in the mature seed it is represented by a layer or two surrounding the radicle in a cap-like fashion. In these persisting cells a large number of starch grains are deposited (Figs. 48 and 74).

Testa

The early stages in the development of the testa have been studied only in *Portulaca quadrifida*, while the later stages have been studied in all the three species described in the paper. It has been shown previously that the integuments consist mostly of two layers of cells throughout their length (Fig. 49). Out of these four layers only three layers persist in the mature seed to form the testa. Two outer layers belong to the outer integument, while the third layer belongs to inner integument and is the inner layer. The outer layer of the inner integument is destroyed during the course of development (Figs. 50 and 75).

In the three layers that persist in the seed, some small grains first begin to be deposited in the outermost layer (Fig. 49). The

deposition of these grains begins some time before fertilisation. Similar grains are also deposited in the innermost layer (Fig. 50). The grains deposited in this layer are small in the beginning. The deposition starts generally from the micropylar end and extends towards the chalazal region. As more grains accumulate they fuse to form bigger ones and ultimately the cells are completely filled with them in the mature testa. But before this happens a marked change takes place in the cells of the outermost layer. The shape of these cells in the beginning is shown in Fig. 49 and they are somewhat cubical. Their nucleus does not occupy any fixed position. During the development of the seed, as the grains accumulate, the outer walls of the cells begin to bulge out. The nucleus now shifts into these convex outgrowths and the protoplasm also for some time is present mostly towards this side of the cells (Fig. 50). Meanwhile the deposition of the grains continues and the cells become entirely filled with these grains. The outer convex wall of the cells becomes greatly thickened. When the cells become completely filled up with these grains, the latter fuse to form a homogeneous mass in the mature testa (Figs. 51 and 75). The presence of similar structure in the outer layer of the testa was observed in *Portulaca grandiflora* also (Fig. 75) and the cells during their development undergo a similar series of changes. In *Portulaca oleracea* the cells of the outermost layer do not show any such difference between the peripheral and the remaining part of the cells, but are completely filled with the homogeneous mass of grains. The middle layer of the testa consists of parenchymatous cells in which no deposition of grains takes place even in the mature seed (Fig. 75).

Summary

The development of the anther, ovule, male and female gametophytes and fertilisation in *Portulaca quadrifida* and the development of embryo, endosperm and seed in *P. quadrifida*, *P. oleracea* and *P. grandiflora* has been studied.

The archesporium in an anther-lobe consists of a single row of cells. The primary sporogenous cells directly function as pollen-mother cells. The tapetal cells are of parietal origin and become bi-nucleate. The inner wall of the tapetal and endothecial cells undergoes granular cutinization. The pollen grains are spherical and possess thirty furrows arranged in a pentagonal fashion. The male gametes are definite cells. Starch is present abundantly in the pollen grains. They are shed at the three-nucleate stage.

The ovules are ana-campylotropous. An air-space between the two integuments is seen in the early stages near the chalaza. The epidermal cells of the nucellus just below the micropyle simply stretch out radially, while the surrounding cells undergo periclinal divisions. The primary archesporium consists of a single hypodermal cell. A parietal cell is cut off. The megaspore-mother cell forms a linear or sometimes a T-shaped tetrad. A normal 8-nucleate embryo-sac develops from the chalazal megaspore. The synergids

are hooked. The antipodals are three small cells. The polar nuclei do not fuse before fertilisation, but lie closely pressed against each other just below the egg. During fertilisation the two polar nuclei fuse simultaneously with a male gamete. The other male gamete fuses with the egg. The pollen tube swells on entering the embryo-sac and generally destroys one synergid during fertilisation.

The first division of the egg is by a transverse wall. The micropylar cell divides transversely to form up to three cells. After the first transverse division of the apical cell the resulting micropylar cell divides again transversely and its daughter cell towards the micropyle may behave again in the same manner. Thus a proembryo of five to seven cells is organized. Three apical cells form the embryo proper. The rest form the suspensor. The most apical cell forms the stem tip and two cotyledons. The penultimate cell forms the hypocotyl and the radicle. The third cell completes the root tip and root cap. The mature embryo is annular. Numerous starch grains are deposited in different parts of the embryo. The suspensor is uniseriate in *P. quadrifida*. In other two species it is multiseriate at the micropylar end.

The nuclear endosperm gradually becomes cellular throughout the embryo sac. In the mature seed it is represented by a layer or two surrounding the hypocotyl and the radicle. The central part of the nucellus persists in the mature seed as perisperm and contains abundant starch.

The testa of the mature seed consists of three layers of cells. In the outer and inner layers some kind of grains fill the cells completely. In the middle layer no such grains are deposited. In *P. quadrifida* and *P. grandiflora* the cells of the outermost layer of the testa prominently bulge out, and due to this the testa becomes warty.

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