STUDIES IN THE CHENOPODIACEÆ

II. Embryology of *Arthrocnemum indicum* Moq.

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Introduction

Arthrocnemum indicum Moq. is a perennial herb with prostrate stem giving out numerous erect or ascending, fleshy, short jointed, leafless branches. The flowers are minute, hermaphrodite, and form cone-like spikes. There is only one stamen with large oblong anther. The seed is trigonous and compressed with thin yellow testa. The plant is a mangrove like *Suæda maritima*. The flowers were collected from Dandi, a village near Surat.

References to previous literature on the family have been given in Part I. These are mainly on *Chenopodium Bonus Henricus* (Souèges, 1920), *Beta vulgaris* (Artschwager, 1927; Artschwager and Starret, 1933; and Oksijuk, 1927), *Kochia scoparia* (Williams, 1932), *Atriplex hymenelytra* (Billings, 1934), *Chenopodium album* (Bhargava, 1936), *Suæda fruticosa* (Mahabale and Solanky, 1953), and on a few other plants of Chenopodiaceæ (Cooper, 1935). A reference to Schnarf (1927) and other literature on the Chenopodiaceæ showed that no work has been done on *Arthrocnemum indicum*. It was, therefore, thought worthwhile to work out its embryology.

The material was fixed in formalin-acetic-alcohol and Nawaschin's fluid, of which the latter gave good results. The customary methods of dehydration and embedding in paraffin were followed. Sections were cut at 10–18 μ thick and stained with iron-hæmatoxylin which proved satisfactory.

Description

Floral organogeny.—The development of floral organs is strictly acropetal as in *Beta vulgaris* (Artschwager, 1927), *Chenopodium album* (Bhargava, 1936) and *Suæda fruticosa* (Mahabale and Solanky, 1953). An individual flower primordium appears as a conical projection (Fig. 1) on which perianth

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soon appears (Fig. 2). Stamen primordium appears next (Fig. 3) followed by carpels (Fig. 4). The carpels grow upwards (Fig. 5) and enclose the ovule (Fig. 6). Usually there is only one stamen but in some cases two.

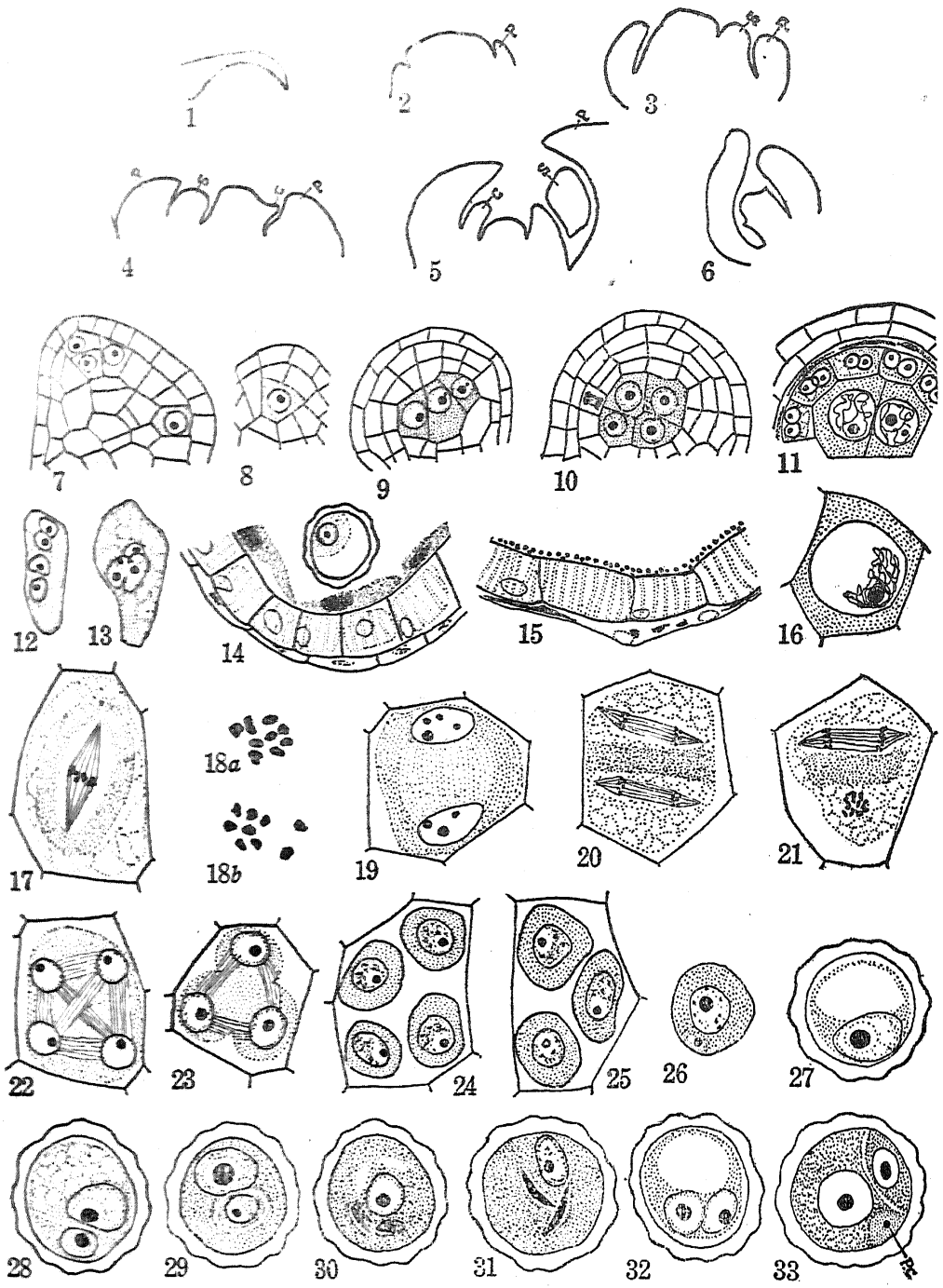
Microsporangium.—As the anther becomes four-lobed, in each lobe one or two hypodermal archesporial cells are differentiated (Fig. 7). The archesporial cells cut off the primary parietal cells on the outer side (Fig. 7). They divide anticlinally first (Fig. 8) and then periclinally to form two layers (Fig. 9). The outer one forms endothecium and the inner divides to give rise to ephemeral middle layer and the tapetum (Figs. 10 and 11). The archesporial cells after cutting off the primary parietal cells become sporogenous. They divide, enlarge and form pollen mother cells.

The tapetal cells are uninucleate but through mitotic divisions become two- to four-nucleate (Figs. 11–12). The nuclei fuse to form a single multinucleolate nucleus (Fig. 13). Four-nucleate condition of tapetal cells has been observed in *Beta vulgaris* (Artschwager, 1927), *Atriplex hymenelytra* (Billings, 1934), *Chenopodium album* (Bhargava, 1936) and *Suæda fruticosa* (Mahabale and Solanky, 1953).

After the formation of microspores, walls of the tapetal cells break down and protoplasts coalesce to form a continuous mass at the periphery of the pollen chamber (Fig. 14) which completely disappears later (Fig. 15). Tapetum is of the secretory type as in *Suæda fruticosa* (Mahabale and Solanky, 1953). The formation of a tapetal periplasmodium is not known in Centrospermales (Schnarf, 1931).

The endothecium becomes fibrous (Figs. 14 and 15). In mature anthers, small peculiar droplet-like bodies appear on the inner surface of the endothecium (Fig. 15). A few of the endothecial cells in the middle of each anther lobe do not get thickened. The epidermis degenerates (Fig. 14) and its remnants are found on the outer wall of the endothecium (Fig. 15).

Microsporogenesis.—The microspore mother cells undergo a period of rest and increase in size. The heterotypic division commences with thickening of reticulum threads (Fig. 11). The threads condense into a typical synizetic knot (Fig. 16). The metaphase spindle at the first division is surrounded by a clear space around which lie a dense cytoplasmic zone and a more vacuolated area (Fig. 17), as in *Chenopodium album* (Bhargava, 1936) and *Suæda fruticosa* (Mahabale and Solanky, 1953). Nine bivalents were counted at the metaphase (Figs. 18 *a* and 18 *b*). The interphase is well defined (Fig. 19). After a brief period of interkinesis the daughter nuclei enter the homotypic division. The spindles of this division may be parallel



TEXT-FIGS. 1–33. *Arthrocnemum indicum* Moq.

Figs. 1–6. Organogeny of the flower, × 132. P—perianth primordium; S—stamen primordium; C—carpel primordium. Figs. 7–11. Stages in the development of the anther, × 331. Fig. 12. A quadri-nucleate tapetal cell, × 705. Fig. 13. A tapetal cell showing fusion of nuclei, × 705. Fig. 14. Anther wall at microspore stage; note the degenerating tapetum, × 331. Fig. 15. Fibrous endothecium; note the degenerating epithelium, × 331. Figs. 16–22. Stages in the division of microspore-mother-cell, × 705. Figs. 18 *a*, 18 *b*. Metaphase

plates showing 9 bivalents. Fig. 23. Cytokinesis by furrowing, × 705. Figs. 24–25. Isobilateral and tetrahedral tetrads, × 705. Figs. 26–27. Uninucleate pollen grains, × 705. Figs. 28–29. Two-celled pollen grains, × 705. Figs. 30–31. Three-celled pollen grains, × 705. Fig. 32. A pollen grain with two vegetative nuclei, × 705. Fig. 33. A pollen grain with a prothallial cell—P.C., × 705.

(Fig. 20) or at right angles to each other (Fig. 21). Secondary spindles connect the four daughter nuclei with one another (Fig. 22).

The original wall of the microspore mother cells remain intact during the whole process of meiosis. Quadripartition occurs by furrowing (Fig. 23). The tetrads may be isobilateral (Fig. 24) or tetrahedral (Fig. 25). Microspores are liberated by the disintegration of the wall of the microspore mother cell.

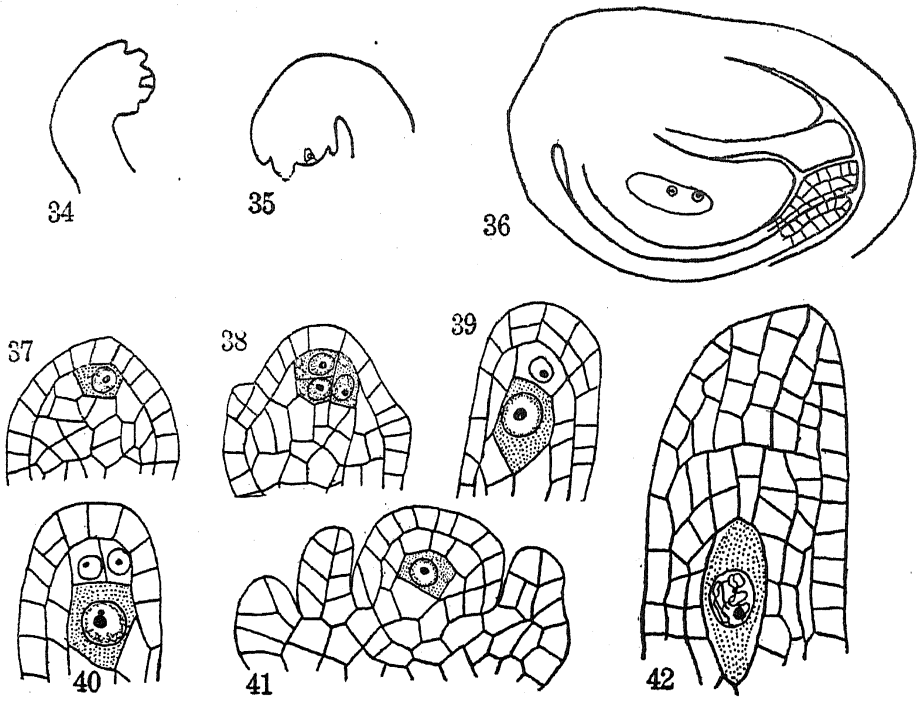
Male gametophyte.—The young microspore when separated from the tetrad has a prominent nucleus surrounded by dense cytoplasm (Fig. 26). As it increases in size its nucleus is pushed to the periphery by appearance of a large vacuole in the centre (Fig. 27). It develops exine with thickenings characteristic of the Chenopodiaceæ and thin intine (Fig. 27). The exine is perforated by oval pores arranged regularly all over.

The divisions of the microspore nucleus results in the formation of a large vegetative and small lenticular generative cell separated by a curved space (Fig. 28). The presence of a definite wall between the two as in some Chenopodiaceous plants (Cooper, 1935) was not noticed. The generative cell leaves its peripheral position and gets embedded in the cytoplasm of the tube cell (Fig. 29). It enlarges and divides to form two male cells which are oval first (Fig. 30), but become elongated and spindle-shaped later (Fig. 31). During all these stages the size of the pollen increases. The pollen grains are tri-cellular at the time of shedding as in *Spinaceæ oleracea* (Tuschnjakowa, 1929), *Chenopodium album* (Bhargava, 1936), and *Suæda fruticosa* (Mahabale and Solanky, 1953). Tri-nucleate pollen grains are characteristic of the whole order Centrospermales.

Abnormalities.—In some pollen grains the nucleus divides and gives rise to two vegetative nuclei (Fig. 32) as in *Chenopodium album* (Bhargava, 1936) and *Suæda fruticosa* (Mahabale and Solanky, 1953). In others there was a prothallial cell (Fig. 33, P.C.). Prothallial cell was also noticed in *Atriplex hymenelytra* by Billings (1934).

Megasporangium.—The crassinucleate bi-tegmic ovule arises as a blunt protuberance on the placenta. To begin with it is erect, but gradually curves (Fig. 34) and gets completely inverted (Fig. 35). Its curvature increases till it takes another turn through an angle of more than 90° (Fig. 36). The

two integuments arise exogenously, only epidermis taking part in their formation. These epidermal cells enlarge, project out, divide and establish apical cells (Fig. 38), which form two-layered integuments. The inner integument arises first followed by the outer one (Figs. 38 and 41). Only the outer



TEXT-FIGS. 34-42. *Arthrocnemum indicum* Moq.

Figs. 34–36. Curvature of ovule, × 147. Figs. 37–42. Megasporogenesis, × 475. Fig. 37. Hypodermal archesporial cell in the nucellus. Fig. 38. Multicellular archesporium. Fig. 39. Megaspore mother cell and primary parietal cell; note the integumentary apical cells. Fig. 41. L.S. Ovule showing two integuments. Fig. 42. L.S. Nucellus showing megaspore mother cell in early prophase and nucellar beak.

integument forms the micropyle (Fig. 36). The integuments are each two layered but the part of the inner integument forming the micropyle is thicker (Fig. 36) as in many families of Centrospermales (Maheshwari, 1945). Occasionally there is a prominent air space between the two integuments in the chalazal region. Such air space was also noticed in *Chenopodium album* (Bhargava, 1936) and *Suæda fruticosa* (Mahabale and Solanky, 1953).

The archesporium is differentiated as a single cell before the integuments (Fig. 37). Multicellular archesporium was also observed (Fig. 38). The archesporial cell divides to form a primary parietal cell and the megaspore mother cell (Fig. 38). The cutting off of a parietal cell was noticed in *Atriplex hymenelytra* (Billings, 1934), *Chenopodium album* (Bhargava, 1936),

Suæda fruticosa (Mahabale and Solanky, 1953), and some other plants of the Centrospermales.

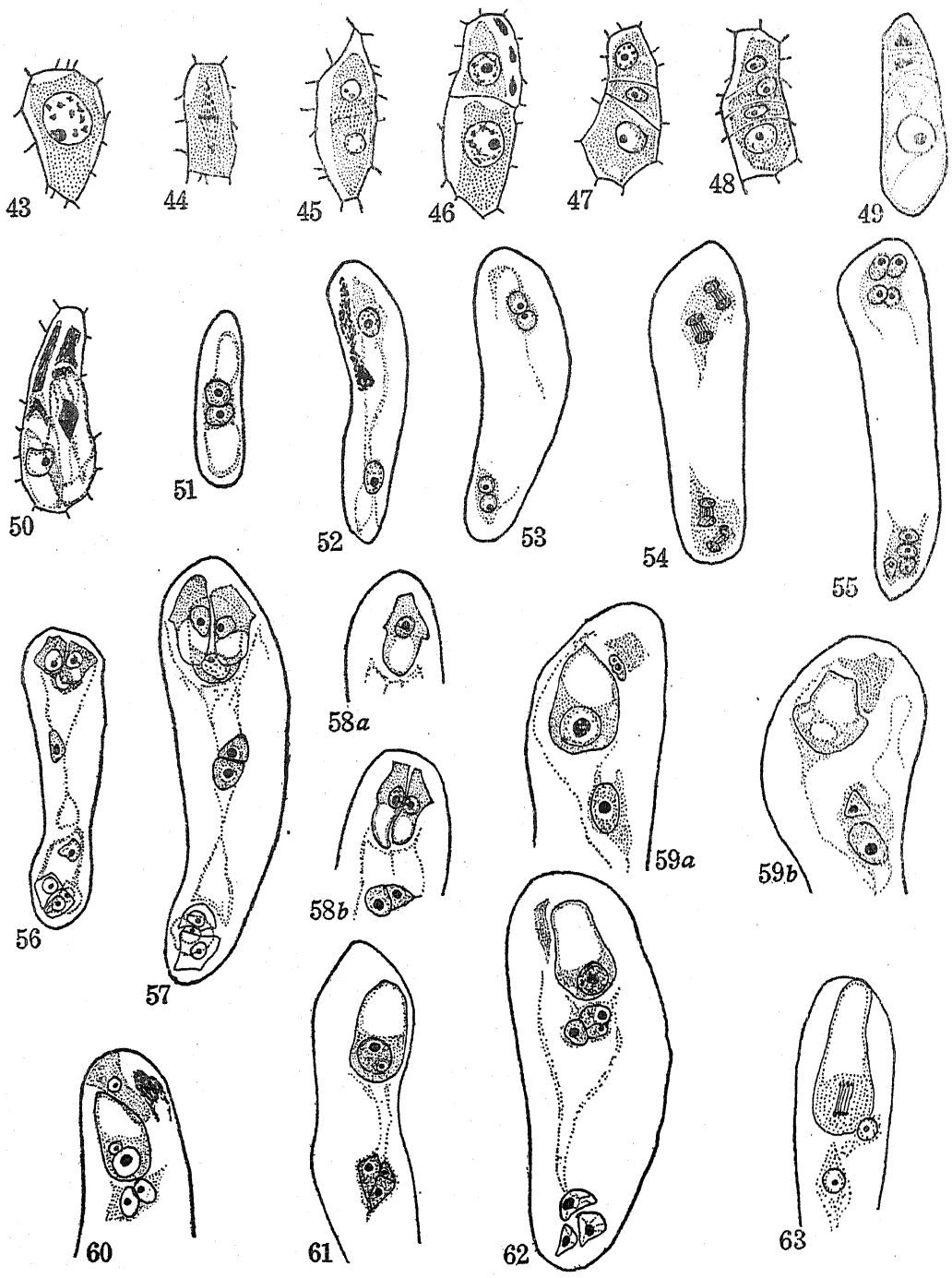
The primary parietal cell divides anticlinally first (Fig. 40) and then periclinally to form a few layers. The epidermis also divides to form nucellar beak (Figs. 39 and 42). The nucellus becomes massive, and the megaspore mother cell gets deep-seated (Fig. 42). The cells of the nucellar beak which are situated directly below the micropyle become narrow and radially elongated as in *Beta vulgaris* (Artschwager and Starrett, 1933), *Chenopodium album* (Bhargava, 1936) and *Suæda fruticosa* (Mahabale and Solanky, 1953).

Megasporogenesis.—The megaspore mother cell undergoes a long period of rest and enlarges (Figs. 39 and 40). It divides meiotically (Figs. 42–45) to form two dyad cells of which the micropylar one is smaller than the chalazal one (Fig. 46). Usually only chalazal dyad cell divides, resulting in a row of three cells (Fig. 47) or rarely both the dyad cells divide again to give rise to a linear tetrad of four megaspores (Fig. 48). Three to four megaspores were observed in *Basella* and *Ullucus* (Rocen, 1927), and three in *Atriplex* (Billings, 1934).

At times two archesporial cells become functional and give rise to double uninucleate embryo-sacs, of which one always degenerates (Figs. 50 and 52) and the other develops.

Embryo-sac.—The chalazal megaspore functions and gives rise to the embryo-sac. The nucleus is located in the centre and there are large vacuoles at both the ends of it in the long direction of the cell (Figs. 49–50). The two daughter nuclei formed after the first division (Fig. 51) reach their respective poles (Fig. 52), and divide again to form two nuclei at each pole (Fig. 53). They further divide (Fig. 54) and form octo-nucleate embryo-sac (Fig. 55). The micropylar nuclei are larger than the chalazal ones. During all these stages the size of the embryo-sac increases, a large central vacuole is formed and the nuclei lie in the scanty protoplasm which collects mostly at poles. The mature embryo-sac is monosporic, eight-nucleate and falls under the *Polygonum* type (Maheshwari, 1950).

Female gametophyte.—From the micropylar group of four nuclei differentiates the egg apparatus and upper polar nucleus, while the chalazal group differentiates into lower polar nucleus and three antipodals (Figs. 56–57). A young synergid has dense cytoplasm without vacuoles and is without hooks (Fig. 56). It develops a prominent hook and a large, basal, vacuole later (Figs. 57–58) as in *Chenopodium album* (Bhargava, 1936) and *Suæda fruticosa* (Mahabale and Solanky, 1953). The egg is broadly flask-shaped.



TEXT-FIGS. 43–63. *Arthrocnemum indicum* Moq.

Figs. 43–48. Megasporogenesis, × 475.—Fig. 43. Megaspore mother cell in diakinesis. Fig. 44. Megaspore mother cell in metaphase. Fig. 45. The same in telophase. Fig. 46. Two dyad cells. Fig. 47. A row of three cells; note that the micropylar dyad cell has failed to divide. Fig. 48. Linear tetrad of four megaspores. Figs. 49–58. Development of embryo-sac.—Fig. 49. Uninucleate embryo-sac. Fig. 50. Double uninucleate embryo-sacs. Figs. 51–55. Two, four and eight-nucleate embryo-sacs. Fig. 56. Embryo-sac showing polar nuclei

migrating towards centre. Fig. 57. The same fully formed. Figs. 58 *a*–58*b*. Hooked synergids and synergid-like egg. Figs. 59–62. Stages in fertilization, × 475.—Figs. 59 *a*–59 *b*. Discharge of male gametes from the pollen tubes. Fig. 60. One male gamete fusing with the egg and the other lying in the pollen tube. Fig. 61. Syngamy and triple fusion. Fig. 62. Triple fusion; note the antipodals. Fig. 63. Division of the zygote; note the free nuclei of the endosperm.

It has a large micropylar vacuole and its nucleus is embedded in the cytoplasm at the chalazal end (Figs. 57–63). The egg always extends beyond the synergids. In one case the egg has a large chalazal vacuole and appeared like a synergid (Fig. 58).

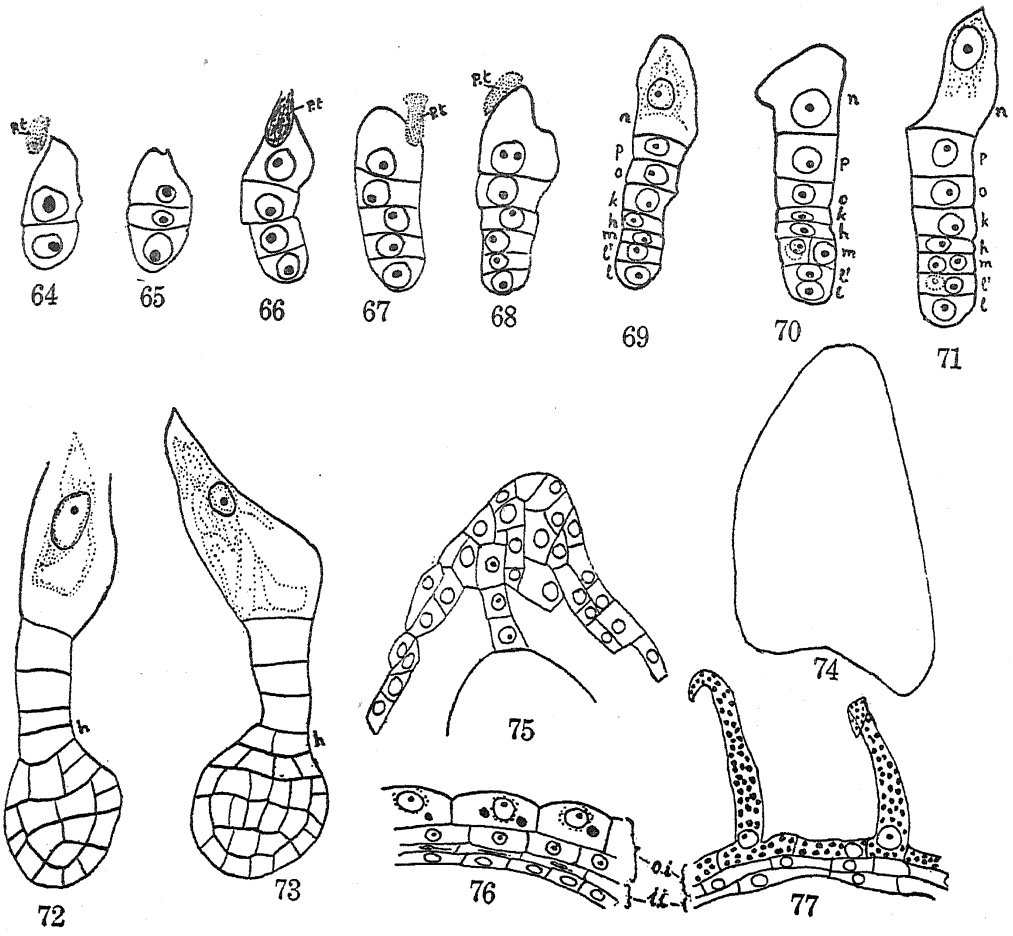
The polar nucleus from each pole migrates towards the centre of the embryo-sac (Fig. 56) where it meets its complement (Fig. 57). They enlarge in size but do not fuse before fertilization. The antipodals are seen upto the time of fertilization (Fig. 62), after which they degenerate. Usually they form a triangle, one above and two below (Figs. 56, 57, 62).

The embryo-sac when fully formed is spindle-shaped (Fig. 62). At first it is included in the micropylar end of the nucellus, but after fertilization it becomes elongated and curved by absorption of nucellar cells at the chalazal end.

Fertilization, endosperm and embryo.—The pollen tube enters the ovule through the micropyle and passing through the nucellar beak enters the embryo-sac. Here it enlarges and discharges the male cells (Figs. 59 *a* and 59 *b*). One male gamete fuses with the egg and the other with the two polar nuclei. Various stages in their fusion were observed (Figs. 59–62). The pollen tube is persistent and can be seen upto six-celled stage of pro-embryo (Figs. 66–68, *p.t.*).

The primary endosperm nucleus divides before the fertilized egg (Fig. 63). The endosperm is free nuclear, but later becomes cellular throughout the embryo-sac. The developing embryo absorbs greater part of the cellular endosperm and only a few layers of endosperm remain ensheathing the radicle of the embryo (Fig. 75) as in *Beta vulgaris* (Artschwager, 1927) and *Chenopodium album* (Bhargava, 1936).

The zygote undergoes a transverse division (Fig. 63) forming an apical and a basal cell (Fig. 64). The apical cell divides to form a row of three cells (Fig. 65); or both the cells divide simultaneously to form a four-celled pro-embryo (Fig. 66). By further divisions eight-celled pro-embryo is formed (Figs. 67–70). The cells can be designated from below upwards as *l*, *l'*, *m*, *h*, *k*, *o*, *p*, *n*. The embryo arises from *l*, *l'*, *m* and *h*. *k*, *o*, *p* form uniseriate suspensor while *n* enlarges, pushes itself in the nucellus at the micropylar end, and forms a haustorial cell (Figs. 71–73). Haustorial cell was



TEXT-FIGS. 64–77. *Arthrocnemum indicum* Moq.

Figs. 64–75. Stages in the development of embryo, × 311; note the development of the haustorial cell in Figs. 68–73 and the cellular endosperm in Fig. 75. Figs. 76–77. Development of the testa, × 220.

also observed in *Beta vulgaris* by Oksijuk (1927). At seven- or eight-celled stage of the pro-embryo *m* divides by a vertical wall and is followed by a vertical partition in *l'* (Fig. 71). The division of *l* lags behind. Dermatogen, periblem and pleurome are differentiated later (Figs. 72, 73). Division of *h* takes place very late (Fig. 73). Fig. 74 shows embryo with two cotyledons marked out. The suspensor remains uniseriate (Fig. 75). Uniseriate suspensor is also present in *Kochia scoparia* (Williams, 1932), while in *Chenopodium Bonus Henricus* (Soueges, 1920), *Beta vulgaris* (Artschwager, 1927), *Chenopodium album* (Bhargava, 1936) and *Suæda fruticosa* (Mahabale and Solanky, 1953) it is massive. Mature embryo is spiral as in many Centrospermales.

Seed coat.—The cells of both integuments are alike but later undergo some changes. In the cells of the outer layer of the outer integument large

taniferous granules are deposited. They bulge out and develop into unicellular hook-like hairs. The cells of the outer layer of the inner integument gradually disappear. The inner layers of the outer and inner integuments persist (Figs. 76–77, *i.i.*, *o.i.*).

Summary

1. The embryology of *Arthrocnemum indicum* Moq., a mangrove was studied. The floral parts develop in acropetal succession.

2. Archesporium is hypodermal in origin. The endothecium become fibrous. Middle layer is ephemeral. Tapetum is of the secretory type. The tapetal cells become bi-quadri-nucleate. Their nuclei fuse.

3. Nine bivalents were counted for the first time. Cytokinesis is by furrowing. The pollen-mother-cell-wall dissolves after the formation of microspores.

4. The reproductive cell is separated from the tube cell by a distinct space. The pollen grains are tri-cellular at the time of shedding. Some pollen grains had two vegetative nuclei while some formed a prothallial cell.

5. The ovule has two integuments, each one of which is two-layered. The inner only forms the micropyle and is thicker at the tip. An air space is present between the two integuments at the chalazal end. The nucellar epidermis and primary wall cells divide to form massive nucellus.

6. The archesporial cell in the nucellus is hypodermal and cuts off a primary parietal cell. Multicellular archesporium was also observed. Usually the division of the micropylar dyad cell is suppressed. Rarely tetrads of four megaspores are formed.

7. The chalazal megaspore is functional. The embryo-sac is monosporic and of *Polygonum* type. Synergids are hooked. Egg is flask-shaped and hangs lower than the synergids.

8. Various stages in syngamy and triple fusion were observed.

9. Endosperm is free nuclear but becomes cellular later throughout the embryo-sac. In mature seed only some layers of endosperm remain just above the radicle.

10. Development of the embryo was worked out. The suspensor is uniseriate. A single large haustorial cell is present at the base of the suspensor.

11. Testa has three layers of cells. The outer layer of the outer integument forms unicellular hairs.

In conclusion, we have to thank Prof. P. Maheshwari for some suggestions and literature.

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