

A CONTRIBUTION TO THE LIFE-HISTORY OF *SPHENOCLEA ZEYLANICA* GAERTN.

By S. B. KAUSIK AND K. SUBRAMANYAM
(Department of Botany, Central College, Bangalore)

Received April 8, 1946

(Communicated by Prof. L. Narayan Rao)

THE earlier literature relating to the embryological features of the Campanulaceæ has been reviewed by Schnarf (1931), and the recent work has been described by Kausik and Subramanyam (1945) in their paper on the embryological study of *Isotoma longiflora* Presl. *Sphenoclea zeylanica* Gaertn., a monotypic species belonging to this family, has been selected for the present investigation. It is found in moist districts, in swampy places, especially near the coast. It is a stout herb reaching one to three feet in height with oblong-lanceolate, glaucous green leaves. The greenish-yellow flowers are found in terminal close spikes. Fruit is a membraneous depressed globose-capsule with the upper portion of the capsule opening out in the form of a lid.

MATERIAL AND METHODS

The material for the present study was collected at Guddancherry, a suburb of Madras. It was fixed in formalin acetic alcohol and Allen's modified Bouin. At 70% stage in dehydration the ovaries were placed in Carnoy's fluid for half an hour to facilitate slight hardening of the material and thus preventing the detachment of the ovules from the placenta. Sections were cut ten to twenty μ in thickness and stained in Heidenhain's iron-alum hæmatoxylin with eosine as counterstain for contrast.

MICROSPORANGIUM

The wall of the young anther (Fig. 1) shows three layers below the epidermis ; of these the outer is the endothecium and the inner the tapetum, with a single middle layer in between. The endothecium shows fibrillar thickenings when the anther is mature, while the middle layer disorganises, and the tapetum, which is uninucleate to start with, becomes binucleate later. At the same time, the tapetal cells are also conspicuously vacuolate (Fig. 1). The microspore mother cells undergo the usual reduction divisions and form the tetrads of microspores. The separation of the microspores is brought about by cleavage furrows (Fig. 3) which start at the periphery. The pollen grains are arranged in a tetrahedral, or sometimes, in an isobilateral manner (Fig. 2). The mature pollen grain is trinucleate (Fig. 4), with a thick rigid

outer exine and a thin delicate intine. Three germ pores are seen in the wall of the pollen grain.

ORGANOGENY OF THE FLOWER

In the organogeny of the flower the different floral parts take their origin in an acropetal succession, the order being, the sepals, the petals, the stamens and finally the carpels.

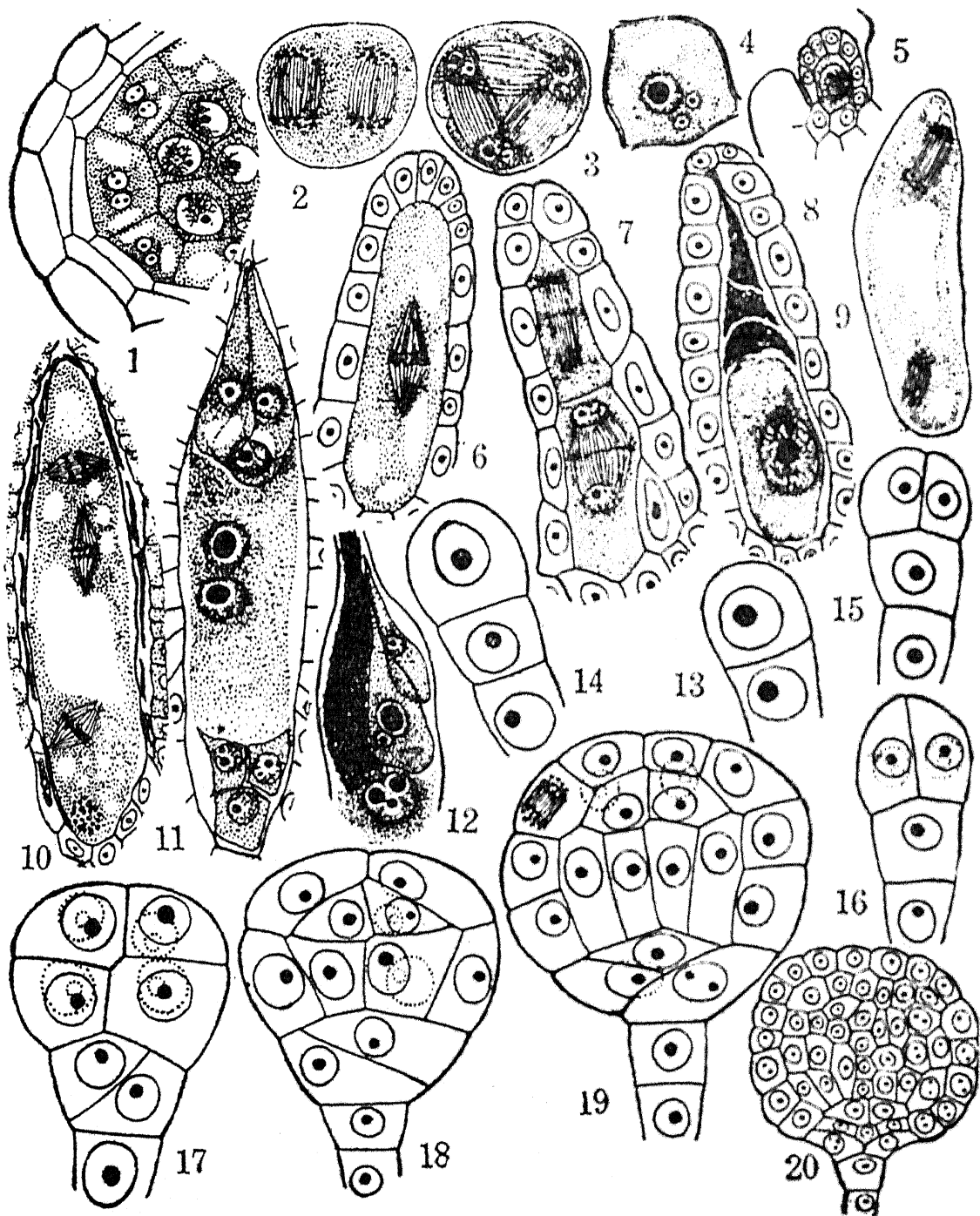
MEGASPORANGIUM AND DEVELOPMENT OF THE EMBRYO-SAC

The ovary is semi-inferior, bicarpellary, syncarpous and bilocular, with an indefinite number of anatropous ovules attached on a massive axile entire placenta. The ovules have a massive single integument which encloses a small nucellus. The hypodermal archesporium functions directly as the megaspore mother cell (Fig. 5). It undergoes the usual reduction divisions and forms a linear tetrad of megaspores (Figs. 6 and 7) of which the upper three megaspores degenerate and the chalazal one enlarges (Fig. 8).

The three nuclear divisions in the chalazal megaspore are quite normal (Figs. 9 and 10) and an eight-nucleate embryo-sac is thus formed according to the normal type. When the second and third divisions in the embryo-sac are in progress the epidermal cells of the nucellus are destroyed. Consequently, the inner epidermis of the integument comes to lie in direct contact with the embryo-sac and forms the integumentary tapetum (Figs. 10 and 11). The fully formed embryo-sac is elongated and straight (Fig. 11). The synergids are long and show pointed apices, with their nuclei situated in the centre. The two polar nuclei meet just above the centre of the embryo-sac and fuse at a later stage to form the secondary nucleus. The three antipodals are organised as definite cells. They degenerate at about the time of fertilisation, or during the early stages of endosperm development (Figs. 21, 22 and 23). The pollen tube enters the embryo-sac by destroying one of the synergids. Syngamy and triple fusion are seen (Fig. 12).

ENDOSPERM AND MATURE SEED

The development of the endosperm is *ab initio* cellular. The first division of the primary endosperm nucleus takes place very much earlier than that of the fertilised egg. It divides in the upper one-third region of the embryo-sac, and is followed by a transverse wall to form a small upper primary micropylar chamber and a large lower primary chalazal chamber (Fig. 21). The next division then follows in both these chambers by means of vertical walls, that in the upper being earlier than the one in the lower (Figs. 22 and 23). Sometimes, however, the chalazal chamber shows the formation of a transverse wall, instead of a vertical one (Fig. 24). After the four-celled



Phenoclea zeylanica Gaertn. (Figs. 1-20). Fig. 1. Portion of a transverse section of a young anther showing wall layers, binucleate tapetum and microspore mother cells. $\times 1260$. Figs. 2 & 3. Second division in microspore tetrad formation. $\times 2700$ each. Fig. 4. A mature trinucleate pollen grain. $\times 2700$. Fig. 5. Megaspore mother cell. $\times 900$. Fig. 6. Metaphase division in the megaspore mother cell. $\times 1800$. Fig. 7. Second division in the formation of the linear tetrad. $\times 1800$. Fig. 8. Enlarging chalazal megaspore and the degenerating upper three megaspores. $\times 1800$. Figs. 9 & 10. Second and third nuclear divisions in the formation of the embryo-sac; note the degenerating nucellar epidermis and the formation of the integumentary

tapetum. $\times 1800$ each. Fig. 11. Fully organised embryo-sac showing the egg apparatus, antipodal cells and the two polar nuclei. $\times 1800$. Fig. 12. A stage in double fertilisation showing remnants of the pollen tube. $\times 1800$. Fig. 13. Two-celled proembryo. $\times 1800$. Fig. 14. Three-celled proembryo. $\times 1800$. Fig. 15-17. Stages in the formation of the octant stage of the embryo. $\times 1800$ each. Fig. 18. The separation of dermatogen by periclinal walls. $\times 1800$. Figs. 19 & 20. Later embryos showing the differentiation into dermatogen, periblem and plerome. In Fig. 19 note the formation of hypophysis by two interesting oblique walls. Fig. 19 $\times 1800$, Fig. 20. $\times 900$. (Original magnifications are indicated here, but the figures have been reduced to two thirds in reproduction).

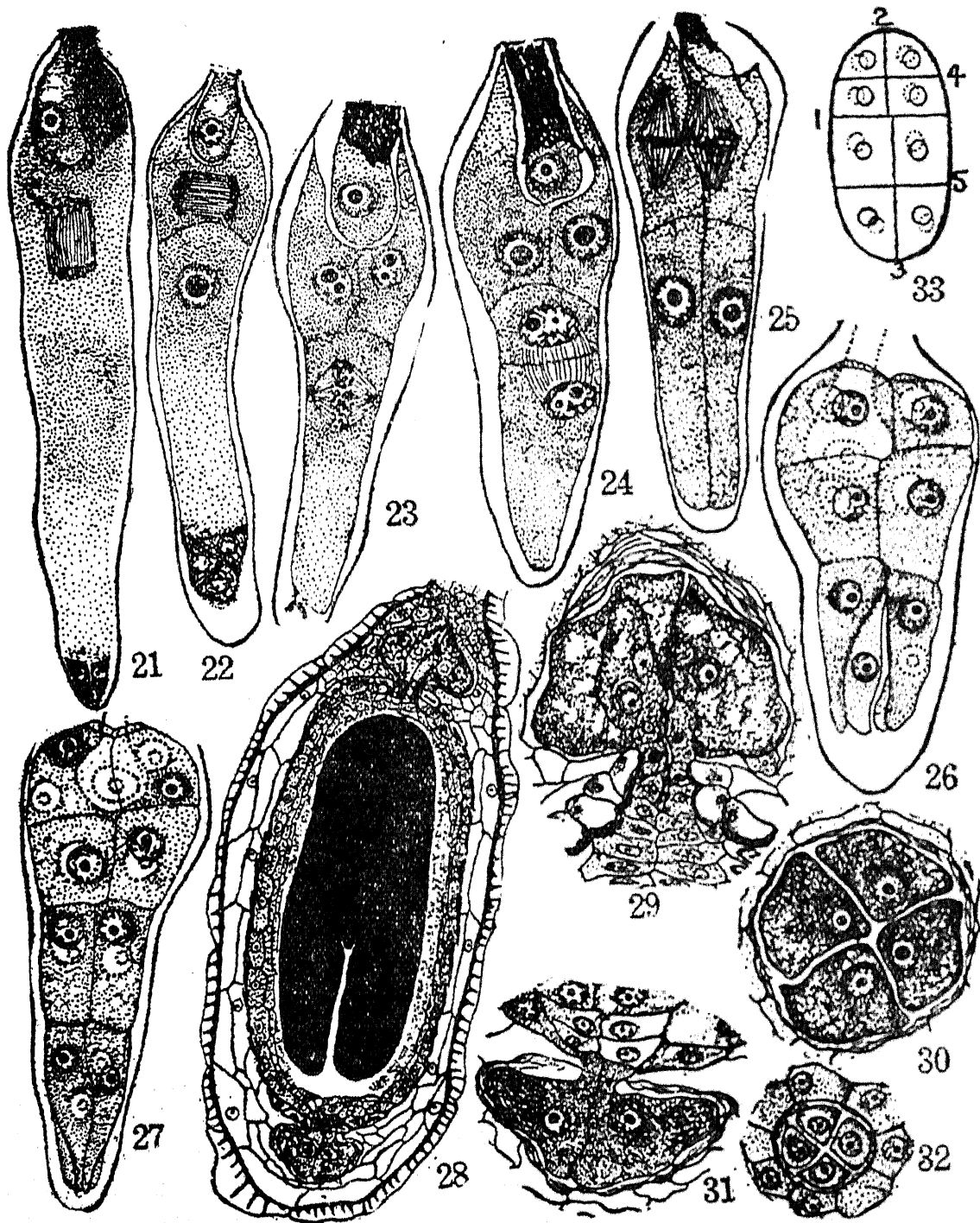
stage subsequent divisions occur in all the cells followed by transverse walls, as well as by a second set of vertical walls at right angles to the first vertical walls in the embryo-sac (Figs. 25 and 26). We find, therefore, that the endosperm, at this stage, is made up of four tiers of cells, with four cells in each tier (Fig. 27). Of these, the micropylar and chalazal tiers are set apart to form the micropylar and chalazal haustoria respectively, while the remaining two central tiers contribute, by further repeated divisions, to the endosperm tissue. The course of development is thus according to the *Scutellaria*-type of Schnarf (1931), with this difference that the primary tiers have four cells each instead of only two.

Both the haustoria are fairly aggressive structures and consists each of four very conspicuous uninucleate cells (Figs. 30 and 32) with dense cytoplasm. In later stages the cells of the micropylar haustorium (Figs. 29 and 30) become quite characteristic with their outer sides bulging prominently and the distal ends tapering to fine points. The haustorium lies in the midst of a rich nutritive tissue in the integument near the micropylar region and remains active for a long period. The chalazal haustorium (Figs. 31 and 32) becomes more or less bulbous and appears like a pad pressed against the rich nutritive cells found at the chalazal region of the seed. Its activity seems to come to an end earlier than that of the micropylar haustorium, for, in an almost ripe seed it appears to be lodged in a compactly collapsed, and doubtless, an erstwhile rich nutritive tissue.

Between these two haustoria the entire portion of the seed is occupied by the mass of endosperm tissue, lying in which is a large dicotyledonous embryo (Fig. 28). The endosperm cells have conspicuous nuclei and show granular contents. The inner walls of the epidermal cells in the mature seed are thickened and show radial spine-like outgrowths, while the outer wall remains thin (Fig. 28).

EMBRYO

The zygote elongates rapidly and becomes tubular. The first division of the zygote-takes place by a transverse wall (Fig. 13). By one more transverse division a small filamentous proembryo of three cells is formed (Fig. 14).



Sphenoclea zeylanica Gaertn. (Figs. 21 to 33), Fig. 21. First division of the primary endosperm nucleus. $\times 1800$. Figs. 22-27. Stages in the development of the endosperm and the separation of the micropylar and chalazal haustoria. $\times 1800$ each. Fig. 28. Longitudinal section of a mature seed showing the remnants of the endosperm filled with granular contents, the micropylar and chalazal haustoria and the mature dicotyledonous embryo. $\times 200$. Fig. 29. The four-celled micropylar haustorium at a late stage in the seed. $\times 900$. Fig. 30. The same

in transverse section to show the four uninucleate cells of the micropylar haustorium. $\times 900$.
Fig. 31. Two of the cells of the chalazal haustorium in a late stage of the seed. $\times 1800$.
Fig. 32. The four uninucleate cells of the chalazal haustorium as seen in transverse section $\times 1260$. Fig. 33. Diagrammatic scheme showing endosperm development. (Original magnifications are indicated here, but the figures have been reduced to two-thirds in reproduction.)

The terminal cell of the proembryo is the embryonal cell, which, after the first vertical (Fig. 15) and the second and third transverse and vertical walls (Figs. 16 and 17) forms the octant stage. With further growth the embryo becomes large and spherical in which the three histogens, namely, the dermatogen, periblem and plerome become differentiated (Figs. 18, 19 and 20). At about the octant stage two intersecting oblique walls are laid in the second cell of the proembryo (Figs. 17, 18 and 19) to form a group of three cells which constitutes the hypophysis. The cells divide further and complete the periblem and the dermatogen at the base of the embryo (Fig. 20). The suspensor is usually made up of two cells. The mature embryo is straight and dicotyledonous, the stem tip arising in the deep notch between the two cotyledons (Fig. 28).

SUMMARY

The wall of the anther shows three layers below the epidermis. The endothecium is fibrillar and the tapetal cells are binucleate. The pollen grain at the time of shedding is trinucleate.

The ovary is semi-inferior and has numerous anatropous ovules on a massive, axile, entire placenta. The ovules have a single thick integument, the innermost layer of which forms an integumentary tapetum. The embryo-sac is formed according to the normal type. The synergids show pointed apices, with the nuclei situated in the centre. The antipodals are organised as definite cells. The two polar nuclei unite before fertilization in the upper one-third region of the embryo-sac to form the secondary nucleus.

Endosperm is *ab initio* cellular and follows the *Scutellaria*-type of Schnarf. The course of development has been described in detail in the paper. Both the micropylar and chalazal haustoria are prominent structures and each of them consists of four large uninucleate cells containing rich cytoplasm.

The course of development of the embryo has been followed in detail and described in the paper. The formation of the hypophysis takes place by two intersecting oblique walls at the base of the embryo.

In conclusion, we wish to thank Dr. L. N. Rao, Professor of Botany, Central College, Bangalore, for his kind encouragement during the course of

this work. We also wish to thank Mr. S. N. Chandrasekhara Iyer, Systematic Botanist, Coimbatore, for kindly determining the species selected for this study.

LITERATURE CITED

1. Kausik, S. B., and Subramanyam, K. Schnarf, K. .. "An embryological study of *Isotoma longiflora* Presl," *Proc. Ind. Acad. Sci.*, B, 1945, 5, 269-78.
- .. *Vergleichende Embryologie der Angiospermen*," 1931, Berlin.