A CONTRIBUTION TO THE EMBRYOLOGY OF *LOBELIA TRIALATA* BUCH.-HAM.

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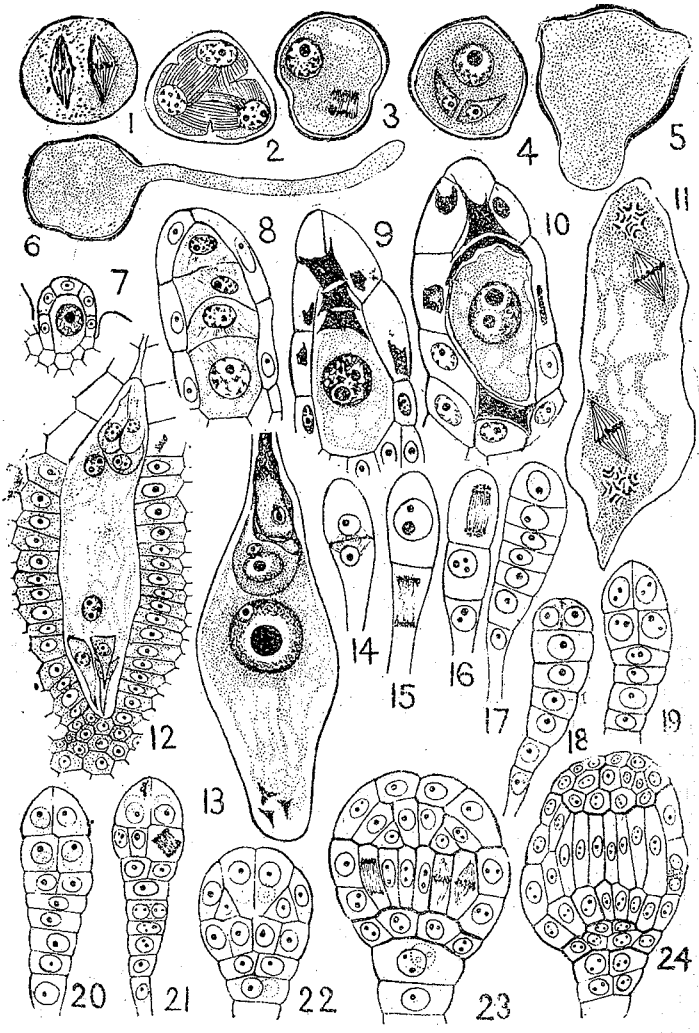
In a recent paper of ours on *Isotoma longiflora* Presl. (1945), we have already mentioned the literature relating to the two closely allied families Campanulaceæ and the Lobeliaceæ. In the present study we have selected for investigation a member of the latter family, *Lobelia trialata* Buch.-Ham. and the results obtained now and in our previous work on *Isotoma longiflora* of the Campanulaceæ have been so encouraging that we feel that a comparative study of these two families from an embryological point of view is likely to prove very interesting and useful. Therefore, it is proposed to investigate the life-histories of as many of the available plants as possible belonging to the families in question.

Lobelia trialata Buch.-Ham. is a glabrous annual growing to a height of fifteen inches, with winged stems and pale blue flowers. The material for study was collected at Nandi Hills, thirty-five miles from Bangalore. The subsequent processes of killing and preparation for study were employed according to the usual methods. To facilitate slight hardening of the flowers and young fruits, at the 70% alcohol stage, they were immersed in Carnoy's fluid for forty-five minutes. Sections were cut 10–24 μ in thickness and stained in Heidenhain's iron-alum hæmatoxylin with eosine as counterstain for contrast.

THE MALE GAMETOPHYTE

The development of the pollen grains is quite normal. In the formation of the pollen tetrads, the two division spindles in the mother cells are arranged either parallel to each other (Fig. 1), or one at right angles to the other (Fig. 2), so that both the isobilateral and tetrahedral forms are met with. The separation of the tetrads takes place by means of peripheral furrows (Fig. 2), which gradually grow towards the centre in the mother cells. The older pollen grains show the two walls clearly (Figs. 3 and 4), the outer thick exine and the inner, rather delicate intine. At this stage were seen in some preparations a large prominent nucleus, the tube nucleus, and a smaller one, the generative nucleus, which was already in the stage of telophase (Fig. 3) during the course of forming the two daughter nuclei which become organised as the two male cells (Fig. 4). The two male cells are especially clear with their cytoplasm sharply marked out from the adjacent mass of the general cytoplasm filling the whole pollen grain.



Figs. 1–24.—Fig. 1. The metaphase spindles arranged in a parallel manner for the second division in the microspore tetrad formation. ×900. Fig. 2. The formation of peripheral furrows between daughter nuclei. ×900. Fig. 3. The large tube nucleus, and the generative nucleus in the telophase stage. ×900. Fig. 4. Pollen grain at the time of shedding showing the large tube nucleus and two small male cells. ×900. Fig. 5. Pollen grain showing three germ pores. Note in one of them the pollen tube has emerged slightly. ×900. Fig. 6. Germination of a pollen grain *in situ* within the anther loculus. ×900. Fig. 7. Portion of an young ovule to show the megaspore mother cell and the integument. ×400. Fig. 8. Second division in the formation of a linear tetrad. ×900. Fig. 9. Enlarging chalazal megaspore and the degenerating upper three megaspores. ×900. Fig. 10. A linear tetrad in which the third megaspore has enlarged. ×900. Fig. 11. Formation of the four-nucleate to the eight-nucleate embryo-sac. ×900. Fig. 12. Fully organized embryo-sac showing the egg apparatus, the polars and the antipodal

cells. Note also the integumentary tapetum. ×630. Fig. 13. A stage showing double fertilization. ×630. Figs. 14–24. Development of the embryo. The primary walls indicated by thicker lines are clearly seen in Figs. 23–24. Figs. 14–21, ×450; Figs. 22 and 23, ×630; Fig. 24, ×450.

There are for each pollen grain three well-defined germ pores (Fig. 5) and in a few anther locules it was seen that the grains had already germinated *in situ* and put out a germ tube through one of the pores. It was, however, rather difficult to make out any contents in such germinating pollen grains and it is, therefore, hard to say anything definite about the fate of such pollen grains which show, peculiarly enough, this precocious tendency in germination. One such pollen grain with a fairly well-developed tube is shown in Fig. 6.

Development of the Embryo-sac

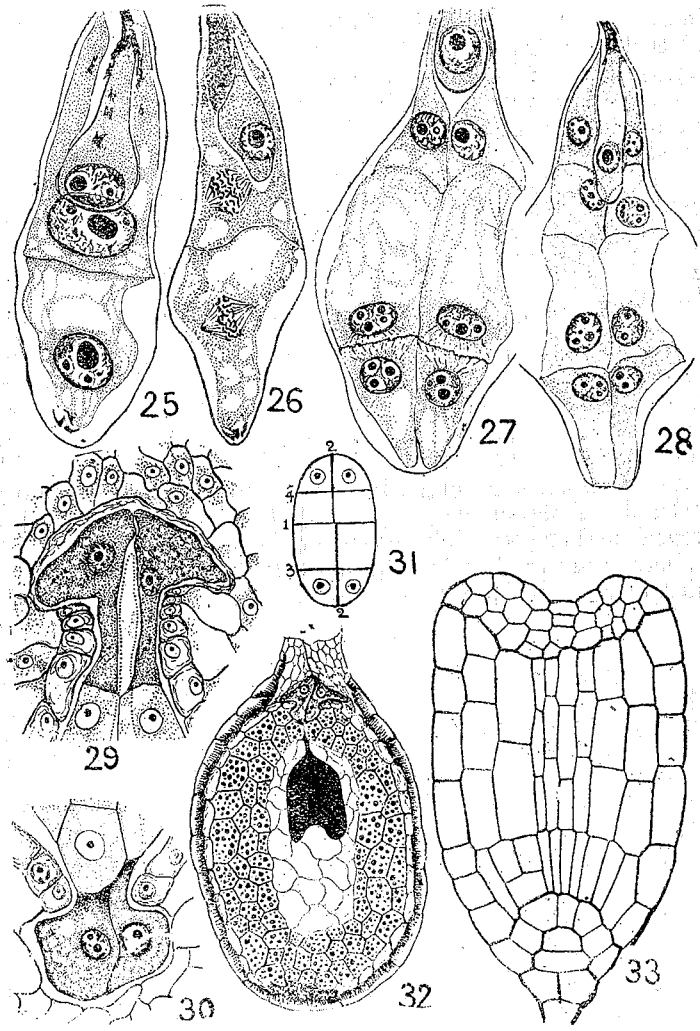
The ovary is bicarpellary and contains a large number of anatropous ovules attached on a central placenta. The ovules arise as small nucellar primordia in which gradually the single massive integument is differentiated. The nucellus is small and shows a large hypodermal archesporial cell which functions directly as the megaspore mother cell (Fig. 7). This latter cell undergoes the usual meiotic divisions and forms a linear tetrad of megaspores (Figs. 8 and 9). Usually the lowest megaspore develops further to give rise to the embryo-sac and the upper three soon degenerate, but occasionally some preparations revealed that the third megaspore may enlarge (Fig. 10). In *Lobelia nicotianæfolia* Heyne, Kausik (1938) has recorded a case where the micropylar megaspore enlarges instead of the usual chalazal one. The subsequent development of the functioning megaspore is normal (Fig. 11), and at the final stage the embryo-sac is typically eight-nucleate. The fully organized embryo-sac is long and tapering at both ends (Fig. 12), and is invested along the sides by the integumentary tapetum. The synergids are pear-shaped and show a small lateral beak just beneath the apex. The two polar nuclei which are at first seen close to the egg-apparatus and the antipodals, migrate later to the centre of the embryo-sac where they fuse immediately to form the large fusion nucleus. The antipodals are organized as definite cells with their lower ends tapering into points and degenerate at about the time of fertilization, when they are seen as darkly stained crushed remnants (Fig. 13).

One of the synergids is destroyed when the pollen tube enters the embryo-sac. Syngamy and triple fusion occur quite normally, the two processes being almost synchronous (Fig. 13).

Embryo

The fertilized egg cell becomes rapidly elongated and the first division of its nucleus takes place followed by the formation of a transverse wall (Fig. 14). Similar transverse walls are laid during subsequent divisions so that finally there results a long and slender proembryo with a number of cells (Figs. 15–17). The first cell of this proembryo then divides by a vertical wall (Fig. 18), which is next

followed by the division of the second proembryonal cell by a similar vertical wall (Fig. 19). Thus a group of four cells is formed and these in turn divide by a second set of vertical walls at right angles to the



Figs. 25–33.—Figs. 25–28. The primary divisions of the embryo-sac in the formation of the endosperm and the haustoria. Figs. 25–27, ×630. Fig. 28, ×450. Figs. 29 and 30. The two-celled micropylar and the chalazal haustoria respectively in an advanced condition. ×450. Fig. 31. Diagrammatic scheme showing endosperm development. Fig. 32. Longitudinal section of a mature seed, showing the dicotyledonous embryo, the starch-filled endosperm, the persisting micropylar and chalazal haustoria and the thick-walled outermost layer of cells. × 120. Fig. 33. A mature dicotyledonous embryo—the thicker lines are the primary walls × 450.

first set, so that now the embryonal mass consists of eight cells (Fig. 20). In the meantime, the third cell of the proembryo also divides by a vertical wall (Fig. 20). It appears that the next one or two proembryonal cells also undergo similar divisions later (Figs. 21 and 22), and thus the embryo is formed not from a single terminal cell of the proembryo, but by quite a number of these cells, so that only a few cells are finally left out to constitute the suspensor.

With the formation, as stated above, of the group of eight cells distally in the proembryo, periclinal walls arise in these cells to form the dermatogen, and still later, after further divisions, the periblem and the plerome are differentiated (Figs. 21–23). At this stage more divisions have taken place in the third embryonal tier (Fig. 24) (derived from the third cell of the proembryo) where, consequently, a group of small cells is formed. The embryo is now large and spherical, and further development proceeds rapidly. Even in later stages when the cotyledons are beginning to grow out, the initial walls of the young embryo forming the different tiers can be clearly seen, and it is, therefore, possible to assign hese tiers to the different primary body regions of the embryo, namely, the cotyledonary tier, also forming the stem tip in the notch between the two cotyledonary lobes, the hypocotyl with its central row of long and narrow plerome cells and the outer zone of much larger periblem cells, and the basal region of the embryo where the radicle is organized (Fig. 33).

ENDOSPERM

The divsiion of the primary endosperm nucleus takes place long before the zygote divides. Following the first division of the nucleus at the centre of the embryo-sac, a transverse wall is laid to form the two primary chambers in the sac (Fig. 25). There is next formed in each of these chambers a vertical wall to form two upper and two lower cells (Fig. 26), and subsequently these pairs of cells become divided transversely, first the lower pair (Fig. 27) and then the upper, so that the mbryo-sac now shows eight cells arranged in four tiers of two cells each (Fig. 28). Of these cells, the uppermost two cells constituting the first tier at the micropylar end of the sac are formed into the micropylar haustorium, and similarly the last pair of cells which makes up the fourth tier at the antipodal end develope into the chalazal haustorium. The remaining four cells belonging to the second and third tiers form the endosperm tissue. The development of the endosperm is thus according to the *Scutellaria*-type of Schnarf (1931), and the sequence of the divisions is shown diagrammatically in Fig. 31.

The micropylar haustorium is two-celled (Fig. 29), each forming laterally a prominent hump and containing a conspicuous nucleus and a finely-vacuolate dense mass of cytoplasm. The haustorium remains active for a long time in the seed (Fig. 32). The chalazal haustorium is also two-celled with prominent contents (Fig. 30). The haustorium is bulbous and seems to stop its haustorial function earlier than the micropylar haustorium. The large mass of endosperm cells filling the cavity of the seed is packed with large grains of starch, except for the

cells lying in the neighbourhood of the developing embryo (Fig. 32). The seed coat is fairly rigid and hard on account of numerous thickenings that are formed in the outermost layer of cells.

CONCLUSION

During the course of the present investigation we have been able to follow in detail certain interesting features in the life-history of this plant. These have already been dealt with in sufficient detail in the foregoing sections of the paper, but to bring together here again the more important facts about them, they are briefly the following. The germination of the pollen grain *in situ* within some of the anther locules appears to be most surprising and interesting, but unfortunately, we are not able to do anything more than merely state this fact and leave out of consideration any discussion on the probable factors that may be responsible for such a condition and also the fate of such germinating pollen grains.

There is nothing unusual in the development of the embryo-sac to merit any further comment here on the observed facts. The formation of the embryo is, however, interesting, and there are, as already described in this paper, important departures from the more normal type where only the terminal cell of a long and filamentous proembryo develops into almost the whole of the embryonal mass. Here it is found that at least the first four cells of the proembryo together contribute to the formation of the embryo, and that further, there is also seen even in very late stages a definite and clear tier-formation corresponding to these initial cells.

The development of the endosperm and the separation of the haustoria conform to the *Scutellaria*-type and it is worthwhile recalling here that a similar course is met with in certain other species belonging to the genus *Lobelia*. According to Hewit's (1939) statement on *L. amœna* the same course is probably met with as already suggested by Maheshwari (1944 *b*) in his note on *L. trigona* Roxb., and we have also very recently come to the same conclusion. It, therefore, appears quite safe to infer that the development of endosperm follows a precise and uniform course in these investigated species of *Lobelia* and is possibly also seen in the other species of this genus. If this should be so, then it becomes quite evident that the constitution of the family Lobeliaceæ as a distinct one from the family Campanulaceæ is, on embryological grounds, quite justifiable as we have already pointed out in our work on *Isotoma longiflora* (1945), which belongs to the Campanulaceæ and where the development shows certain essential departures from the *Scutellaria*-type.

In conclusion, we wish to thank Dr. L. N. Rao, Professor of Botany, Central College, Bangalore, for the many kind courtesies extended to us. We are also thankful to Mr. S. N. Chandrasekhara Iyer, Systematic Botanist, Coimbatore, for kindly determining the species.

SUMMARY

1. The pollen grains at the time of shedding are three-celled. In some anther locules the pollen grains showed germination *in situ*.

2. Megasporogenesis proceeds normally and the embryo-sac is formed according to the Normal-type. Antipodals are formed into definite cells, but these degenerate at the time of fertilization.

3. The fertilized egg develops into a long filamentous proembryo in which the uppermost three or four cells take part in the organization of the embryo. Corresponding to these cells, there is an evident tier-formation even in late stages of the embryo.

4. The primary endosperm nucleus divides much earlier than that of the fertilized egg. After the first transverse wall in the centre of the embryo-sac to form upper and lower primary chambers, subsequent vertical and transverse divisions occur in both the chambers. Thus an eight-celled tissue results, of which the micropylar pair of cells forms a two-celled micropylar haustorium, and likewise the antipodal pair forms a two-celled chalazal haustorium. The development of endosperm corresponds, therefore, to the *Scutellaria*-type of Schnarf.

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