AN EMBRYOLOGICAL STUDY OF *ISOTOMA LONGIFLORA* PRESL

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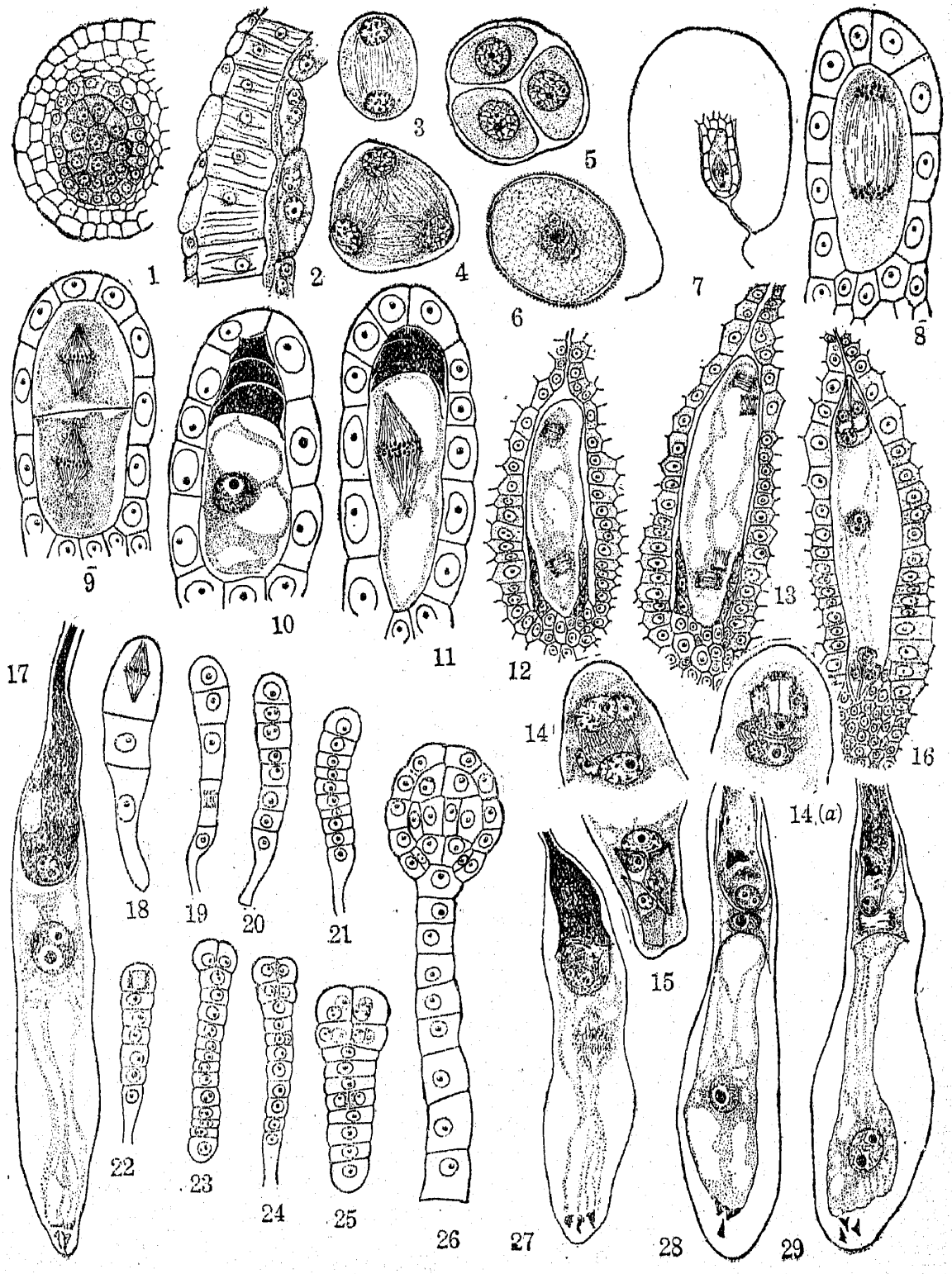
INTRODUCTION

THE earlier literature relating to the embryology of the Campanulaceæ and the related family Lobeliaceæ has been reviewed by Schnarf (1931). Of the recent works mention may be made here of the paper by Rośen (1932) on the embryology of the Campanulaceæ and the Lobeliaceæ, and also of the papers on the Lobeliaceæ by Kausik (1935, 1938), Crete (1938), Hewitt (1939), Cooper (1942) and Maheshwari (1944 *a*, *b*). In this connection, we may note that Cooper's (1942) observations on *Lobelia cardinalis* L. that the synergids and the antipodals take part in the formation of the micropylar and chalazal haustoria respectively have been commented upon as erroneous by Maheshwari (1944 *a*, *b*).

The material selected for the present investigation, *Isotoma longiflora* Presl, is a West Indies species belonging to the tribe Lobelioideæ of the family Campanulaceæ of Engler (1897). This is an erect herb, found in wet situations and growing to a height of two feet, branching sparsely from the base, and possessing plenty of latex in all its parts. Leaves are linear dentate; flowers axillary solitary, or in groups of three, slightly zygomorphic; the corolla is tubular and narrow, and attains a length of about six inches with the limbs spreading horizontally; stamens are five in number with syngenesious anthers having hairs at the tips of connectives; ovary inferior, bicarpellary, syncarpous with indefinite anatropous ovules on a massive central placenta; the fruit is a capsule showing dehiscence by pores at the top.

MATERIAL AND METHODS

The material was collected in the Government Botanical Gardens, Bangalore, and fixed in Allen's modified Bouin. At the 70% stage in dehydration, the old ovaries were placed in Carnoy's fluid for one hour to facilitate slight hardening of the material and thus preventing the detachment of the ovules from the placenta. Subsequent treatment was according to the customary methods. Sections were cut ranging in thickness from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FIGS. 1–29

Figs. 1 to 29.—Fig. 1. Portion of a transverse section of an young anther showing wall layers, tapetum and the microspore mother cells. × 560. Fig. 2. Portion of the anther wall at a much later stage showing the fibrillar endothecium, disorganised middle layer and the binucleate tapetum. × 900. Figs. 3–5. Stages showing the first and second division in microspore tetrad formation; Fig. 3. × 1260, Figs. 4 and 5. × 1800. Fig. 6. A mature trinucleate pollen grain. × 1260. Fig. 7. Young anatropous ovule with massive integument and the first division of the megaspore mother cell. × 540. Fig. 8. Telophase stage in the megaspore mother cell. × 1800. Fig. 9. Second division in the formation of the linear tetrad. × 1800. Fig. 10. Enlarging chalazal megaspore and the degenerating upper three megaspores forming the apical caps. × 1800. Fig. 11. First division in the chalazal megaspore. × 1800. Figs. 12 and 13. Second and third nuclear divisions in the formation of the embryo sac; note the degenerating nucellar epidermis and the formation of the integumentary tapetum. × 900. Figs. 14, 15. Micropylar and antipodal ends of the same embryo sac enlarged to show the late telophase spindles and the organisation of the antipodal cells. × 1800. Fig. 14*a*. Micropylar end of another embryo sac showing the relationship of the daughter nuclei in the organisation of the egg-apparatus. × 1800. Fig. 16. Fully organised embryo sac showing the egg-apparatus, antipodal cells and the fusion nucleus. × 900. Fig. 17. A late stage in fertilization showing remnants of the pollen tube, the zygote nucleus and the primary endosperm nucleus. × 900. Figs. 18–22. Development of the filamentous proembryo; Fig. 18. × 400, Figs. 19–21. × 560, Fig. 22. × 540. Figs. 23–25. Stages in the formation of the octant condition of the embryo; Figs. 23, 24. × 560, Fig. 25. × 800. Fig. 26. Later embryo showing the differentiation into the dermatogen, periblem and plerome. × 900. Fig. 27. First division of the primary endosperm nucleus. 800. Fig. 28. Two-chambered embryo sac. × 800. Fig. 29. Division in the upper chamber. × 800. (Original magnifications are indicated here, but the figures have been reduced to half in reproduction.)

10 to 24 μ. Staining was done in Heidenhain's iron-alum hæmatoxylin with eosine as counterstain for contrast.

Microsporangium

The wall of the young anther (Fig. 1) shows three layers outside the tapetum. These are the outermost epidermis, next the endothecium which acquires fibrillar thickenings later (Fig. 2), and lastly the middle layer which remains single and disorganises at the mature stage of the anther. The tapetal cells are uninucleate to start with, but later become binucleate (Fig. 2). The mother cells undergo the usual reduction divisions and form the tetrads of microspores (Figs. 3–5). The separation of the microspores is effected by cleavage furrows which are initiated at the periphery (Fig. 4). The tetrads are typically tetrahedral (Fig. 5). The mature pollen grains are trinucleate at the time of shedding (Fig. 6) and possess a rigid wall with spinescent projections on the surface. The intine appears as a thin and delicate membrane. Three germ pores are seen in the wall of the pollen grain.

Development of the Embryo Sac

The ovule is anatropous and possesses a single massive integument (Fig. 7) enclosing a small nucellus. The megaspore mother cell undergoes the usual reduction divisions and gives rise to the linear tetrad (Figs. 8–10).

The upper three megaspores soon degenerate, while the chalazal megaspore enlarges to form the embryo sac (Fig. 10). The three nuclear divisions that follow in the functioning megaspore are quite typical, and the eight-nucleate embryo sac is thus formed according to the normal type (Figs. 11–13 and 16). When the second of these nuclear divisions is in progress the epidermal layer of the nucellus is seen to be almost destroyed, except for a few cells at the base, and consequently, during further development, the inner epidermis of the integument comes to lie in direct contact with the embryo sac and becomes differentiated as the integumentary tapetum (Figs. 12, 13 and 16). The final organisation of the embryo sac also proceeds normally with the formation of the egg-apparatus, the three antipodal cells and the two free polar nuclei which fuse almost immediately to form the fusion nucleus (Fig. 16). It may, however, be noted here that the antipodals seem to proceed to organise themselves as cells slightly earlier than the egg-apparatus: Figs. 14 and 15 show the micropylar and antipodal ends respectively of one and the same embryo sac where it is found that the nuclei are still in the late telophase stage at the micropylar end, while the cell formation at the antipodal end is almost completed. Fig. 14*a* represents the micropylar end of another embryo sac where the late telophase spindles of the third nuclear division are seen, with the line of phragmoplasts along the equator quite evident and thus indicating clearly the separation of the daughter nuclei showing their future relationship in the egg-apparatus, namely that the synergids are formed from two sister nuclei while the egg and the upper polar nuclei are similarly formed from the other set of sister nuclei. This is in conformity with the view expressed by Porsch (*cf.* Maheshwari, 1937) with regard to the homology of the angiosperm embryo sac.

In the mature embryo sac, at the time of fertilization, the antipodals are seen as degenerating cells (Fig. 17). The pollen tube enters the embryo sac by destroying one of the synergids. Both syngamy and triple fusion occur as normal processes during fertilization (Fig. 17).

Embryo

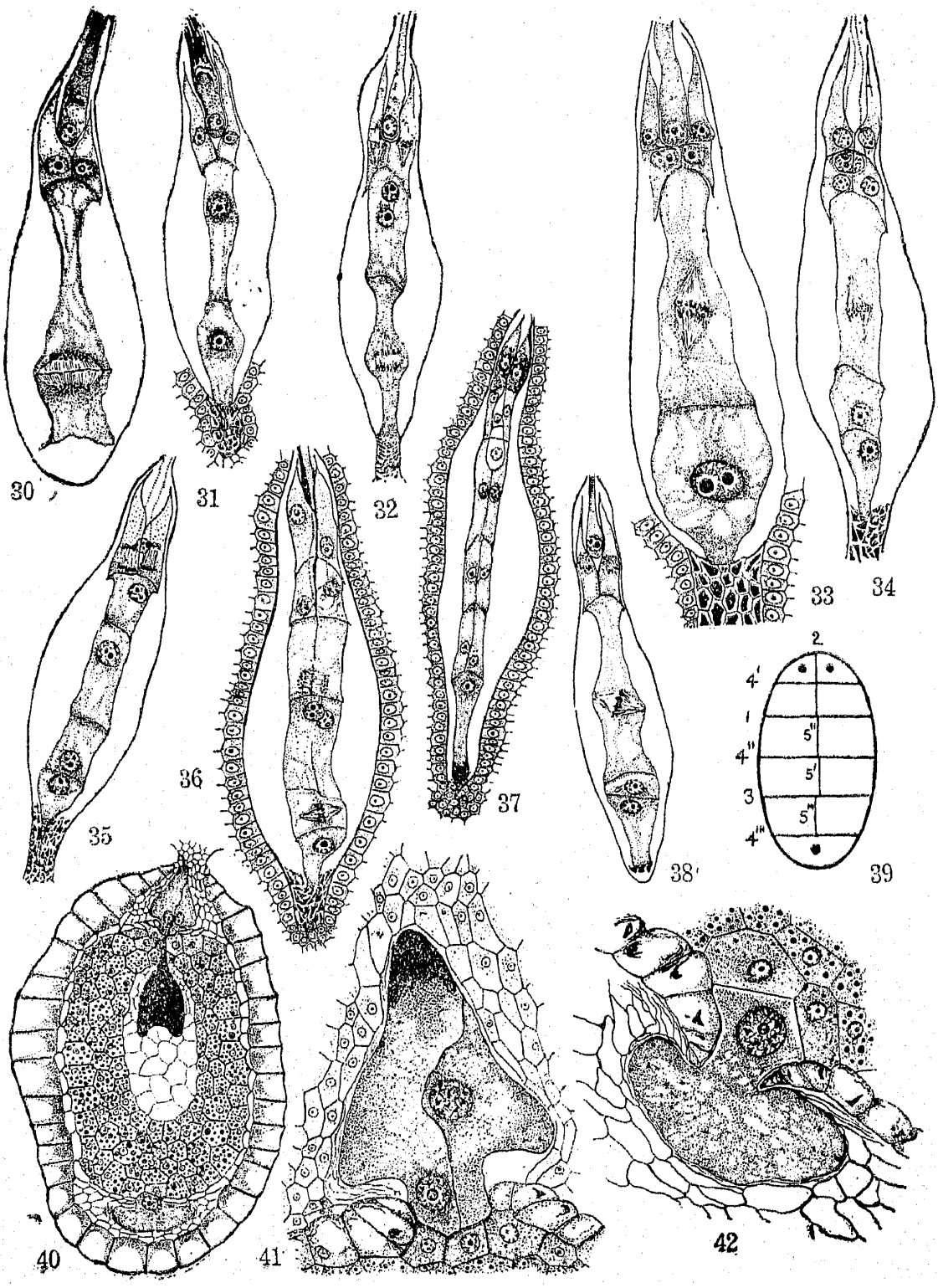
The zygote elongates rapidly and becomes tubular (Fig. 36). The first division in the zygote takes place only after the endosperm has passed through its initial development (Fig. 37). After a series of transverse divisions a filamentous proembryo of 10 to 12 cells (Figs. 18–21) is formed. The terminal cell of this proembryo is the embryonal cell which, after the first vertical, and the second and third transverse and vertical walls, forms the octant stage (Figs. 22 to 25). With further growth, the embryo becomes large and spherical in which the dermatogen, periblem and plerome become

next differentiated (Fig. 26). The first cell of the suspensor becomes wedged into the base of this spherical embryo and gives rise to the hypophysis. The development of the embryo thus conforms to the *Capsella*-type. The late embryo is typically dicotyledonous with the stem tip arising in the deep notch between the two cotyledons (Fig. 40).

ENDOSPERM

The primary endosperm nucleus undergoes the first division in the upper one-third of the embryo sac (Fig. 27). Following this a transverse wall is laid here to form a small upper primary micropylar chamber and a much larger lower primary chalazal chamber (Fig. 28). The upper chamber then divides by a vertical wall to form two cells placed side by side (Figs. 29 to 31) while the nucleus of the lower chamber descends almost to the base of the embryo sac where it divides followed by the formation of a transverse wall (Figs. 28 to 30). Thus a small lower cell and a larger middle cell are formed as shown in Fig. 31, and the embryo sac, therefore, has at this stage three distinct regions. Subsequent divisions take place in all these three regions of the embryo sac, the nuclear divisions being either almost simultaneous in all the regions, or proceeding ahead in certain portions (Figs. 32 to 34). With the completion of these divisions the embryo sac shows six major tiers, the uppermost two consisting each of two cells arranged side by side, and the remaining four tiers having only one cell each (Figs. 34 and 35). The lowest cell in this series does not divide any further, but takes part directly in the formation of the chalazal haustorium which later becomes a prominent bulbous structure with a single large nucleus (Figs. 36, 37, 40 and 42). Similarly, the two cells forming the uppermost tier in the embryo sac also become conspicuous and form the micropylar haustorium, the two cells becoming long and tapering distally, and also later developing a prominent lateral bulge or hump (Figs. 36, 37, 40 and 41). The remaining four central tiers of the embryo sac give rise to the endosperm tissue (Figs. 36, 37 and 40). Here, it is to be noted that it is only the first tier which is two-celled, while the other three are made up of only one cell each (Figs. 34 and 35), but in each of the latter also a vertical wall is next laid so that the primary endosperm cells soon become arranged in two longitudinal rows (Figs. 36 and 37). With further development the endosperm tissue increases rapidly in its bulk and fills the whole cavity of the seed inside the seed-coat (Fig. 40).

A slight departure from the course of development detailed above is met with not infrequently in certain ovules found either in the same ovary or in different ovaries. This is shown in Fig. 38 where it is seen that the nuclear spindle for the division in the large central cell formed after the second



Figs. 30 to 42

Figs. 30 to 42.—Fig. 30. Division in the lower chamber. × 800. Figs. 31–37. Stages in the development of the endosperm and the separation of the micropylar and chalazal haustoria. Fig. 31. × 560, Fig. 32. × 800, Figs. 33–35. × 560, Fig. 36. × 400, Fig. 37. × 560. Fig. 38. A stage in the development of the endosperm according to the *Phyteuma*-type described by Rośen. × 560. Fig. 39. Diagrammatic scheme showing endosperm developement. Fig. 40. Longitudinal section of a mature seed showing the thickened walls of the epidermis, starch-filled endosperm, with the micropylar and chalazal haustoria and the dicotyledonous embryo. × 800. Fig. 41. The two-celled micropylar haustorium at a late stage in the seed. × 900. Fig. 42. The uninucleate single celled and bulbous chalazal haustorium, also at a late stage. × 1260. (Original magnifications are indicated here, but the figures have been reduced to half in reproduction.)

transverse wall at the base of the embryo sac is oriented almost transversely, with the result that a vertical wall is laid here, and not a transverse one as in the method of endosperm development described above. Finally, however, after subsequent divisions in the embryo sac, the primary endosperm cells become arranged in two longitudinal rows as in the first method.

In the mature seed (Fig. 40) the outer epidermis forms a rigid and hard protective covering, with the inner and radial walls of the cells extremely thickened. The endosperm tissue fills the entire seed cavity and its cells are rich in stored starch grains. The micropylar and chalazal haustoria are still very conspicuous and appear as darkly staining structures with prominent nuclei. The embryo with its slender suspensor almost quite shrivelled up lies deeply buried in the large mass of the endosperm tissue.

Conclusions

In the course of the present investigation it has been possible to get a complete and continuous series of stages illustrating endosperm development, and, as already stated, we find that there are two rather distinct and separate types in this development. Both these types are met with equally commonly, occurring as they do each in approximately 50% of the ovules, either in the same or different ovaries. According to the first of these types, which has been dealt with at some length in the paper, the scheme of development, although apparently resembling the *Phyteuma*-type of endosperm development described in the Campanulaceæ by Rośen (1932), appears to differ from this type in certain essential respects in the initial stages. On the other hand, it is to be noted here that the second type referred to briefly in this paper is found to agree closely with the *Phyteuma*-type of Rośen. In view of the fact, therefore, that a course of development of endosperm which seems to represent a marked departure from the *Phyteuma*-type also occurs in *Isotoma longiflora* Presl, we think that it should be referred to a separate, but rather closely related type. Further, it is also met with in quite a large number of observed cases, in fact in about 50% of the ovules. It appears,

therefore, quite reasonable to assume that this scheme of endosperm development is a distinct one which we may designate as the *Isotoma*-type.

Rośen (1932) has also described a second scheme of development in the Campanulaceæ, the *Codonopsis*-type. According to him, an important difference between this and the *Phyteuma*-type is that the chalazal haustorium is two-celled in the former, while it is made up of only a single uninucleate cell in the latter. He further states that the course of endosperm formation according to the *Codonopsis*-type is comparable to that in the *Scutellaria*-type, and that in the *Phyteuma*-type there is seen a combination of the *Scutellaria*- and the *Ericaceæ*-types of development. It is, therefore, interesting to consider here the course of endosperm formation in the closely related family Lobeliaceæ. According to Maheshwari (1944 *b*), in *Lobelia trigona* Roxb. the primary chalazal chamber of the embryo sac divides by a vertical wall after which both the cells thus formed undergo at least one more division by a transverse wall to give rise to the chalazal haustorium* and an upper set of two cells, the latter contributing also to the endosperm tissue along with the cells derived from the divisions of the upper primary chamber. This course would then correspond to the *Scutellaria*-type. Therefore, the relationship between the families Campanulaceæ and the Lobeliaceæ is thus also found to be very close as indicated by the courses of development seen in the members belonging to these two families. Further, as Maheshwari (1944 *b*) also suggests, according to the observations of Hewitt (1939), the type of development in *L. amoena* appears also to be similar to that in *L. trigona*. Hewitt finds, to quote Maheshwari (1944 *b*), that in *L. amoena* "two cells at each end of the *eight-celled* endosperm develop into large micropylar and chalazal haustoria, the remaining four cells developing into the large central mass of endosperm". In yet another species of *Lobelia, L. trialata* Buch-Ham, the same course of development seems to proceed according to some very recent observations (unpublished) that we have now been able to make.

With regard to the systematic position of *Isotoma*, we may note here that according to Engler (1897) it finds a place in the tribe Lobelioideæ of the family Campanulaceæ. According to Hutchinson (1926), on the other hand, the genus is placed in a separate family, Lobeliaceæ, along with the typical genera like *Lobelia, Pratia* and others under the order Campanales. Thus we see that Hutchinson has split the single family Campanulaceæ of

* It may be noted here that it is likely that this particular stage depicting the exact mode of separation of the chalazal haustoriam in *Lobelia trigona* Roxb. was missed by one of us (Kausik, 1935). We now think that a transverse wall probably occurs as stated by Maheshwari (1942 *b*).

Engler into separate and distinct families, the Campanulaceæ and the Lobeliaceæ. Taking the gross external morphological features, the genus *Isotoma* seems to share many taxonomic characters in common with any typical member of the Lobeliaceæ, as for example *Lobelia.* But when we consider the greater details relating to the Embryology of *Isotoma longiflora* Presl, especially the course of endosperm development we think that there is a very close correspondence between this and the forms like *Phyteuma scheuchzeri, Campanula carpathica, C. patula, C. rotundifolia, Specularia speculum* and *Adenophora* sp. studied by Rośen (1932). It may, therefore, be suggested here that *Isotoma* is probably to be regarded as a form linking the typical Lobeliaceous genera with the forms included under the Campanulaceæ, but finding a place, on embryological grounds, in the latter family; and the close relationship between these two families is thus also again very evident.

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

Summary

1. The anther shows a wall with only three layers; the tapetum becomes binucleate; pollen grains are tri-nucleate at shedding stage.

2. The ovary has innumerable anatropous ovules attached on a massive central placenta. Megasporogenesis proceeds normally and the embryo sac is formed according to the normal type.

3. The embryo is formed according to he *Capsella*-type.

4. Endosperm is *ab initio* cellular, and is formed according to the *Phyteuma*-type described by Rośen.

5. A slight departure from the above type is met with in a large proportion of ovules, and since this is also quite a distinctive one, it may be regarded as a type, the *Isotoma*-type.

6. A reference to the systematic position of the genus *Isotoma* is made in the paper.

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