J. Indian bot. Soc. **60** : 58-64, 1981.

STUDIES ON THE DEVELOPMENTAL ANATOMY OF RANALES V. LATE EMBRYOGENY AND HISTOGENESIS IN *DELPHINIUM*, *NIGELLA* AND *RANUNCULUS*[1]

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ABSTRACT

Late embryogeny has been reported in *Delphinium ajacis* Linn., *Nigella sativa* Linn. and *Ranunculus sceleratus* Linn. In all three species the root pole gets organized first. In *Nigella* a solid core of procambium is observed while in the other two species pith is present in the centre at the mature embryo stage.

INTRODUCTION

Johansen (1950) and Esau (1953) emphasized the need for investigating late embryogenesis with particular reference to organization of primary meristematic tissue systems for a better understanding of histogenesis. Only a few studies on histogenesis in the embryo have emphasized the ontogeny of apical meristems (Schopf, 1943 ; Allen, 1947a, b ; Reeve, 1948 and Mahlberg, 1960). In recent years Pillai and Sukumaran (1969) and Pillai *et al.*, (1975) have dealt with embryogeny and histogenesis in *Cyamopsis tetragonoloba* and *Arachis hypogea* respectively. There are no reports on late embryogeny in Ranales. The present investigation forms part of a wider study on different developmental aspects of Ranales.

MATERIALS AND METHODS

Developing seeds of *Delphinium ajacis*, *Nigella sativa* and *Ranunculus sceleratus* were fixed at definite intervals in FAA. Mature embryos were dissected out from seeds and fixed. The usual methods of dehydration, infiltration and embedding were followed using TBA series. Sections cut at 8 to 10 μ were stained with tannic-acid ferric chloride in conjunction with safranin and light green and also with chlorazol black E.

OBSERVATIONS

The terms globular stage, heart-shaped stage, torpedo stage and mature stage of the embryo have been used following Miller and Wetmore (1945), Mahlberg (1960) and Pillai and Sukumaran (1969).

Early globular stage : The embryo embedded in the cellular endosperm shows a 5-celled suspensor in *Delphinium* and *Nigella*, the embryonal end of the suspensor showing two tiers (Figs. 1, 9). In *Ranunculus* also a suspensor is noticed. The embryo and suspensor together measure about 178 μm in *Delphinium* and 126 μm in *Nigella*. The embryo proper consists of a group of isodiametric, densely stained cells. The outermost cell layer is distinct with predominantly anticlinal divisions. This may be designated as the "embryonic protoderm". The inner cells divide in all planes (Fig. 23).

1 Accepted for publication on June 14, 1980.

The authors are grateful to Prof. S. K. Pillai for going through the manuscript and to Dr. D. Singh, Head of the Department for facilities. One of us (KYK) sincerely acknowledges the award of the Post-Doctoral Fellowship from the Council of Scientific and Industrial Research, New Delhi.

Late globular stage : The embryo is about 210 μm long in *Delphinium* and 259 μm in *Nigella*. Greater frequency of cell divisions in that half of the embryo away from the suspensor end results in the formation of an ovoid embryo with a narrower suspensor end.

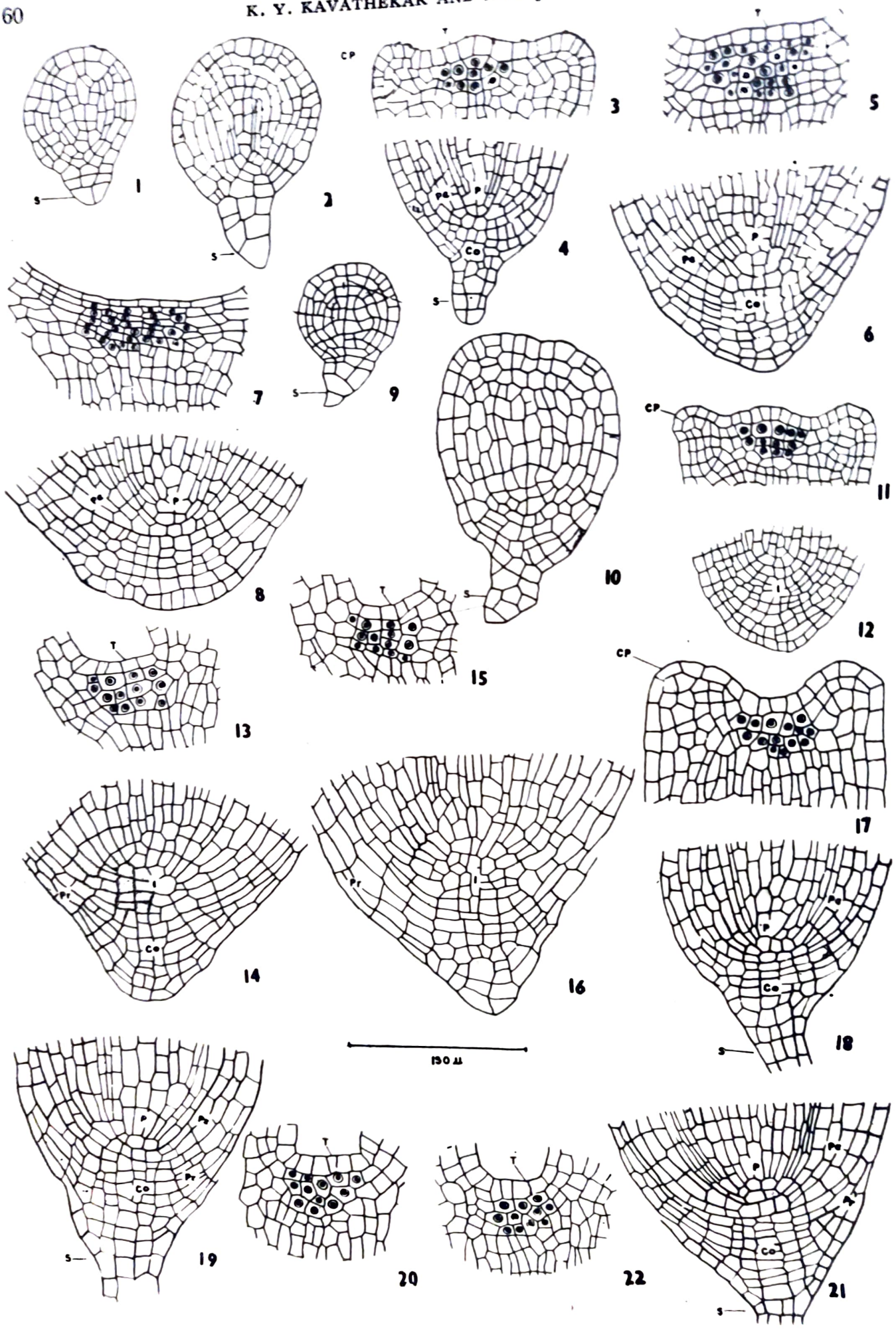
In all the three species, of the primary meristematic tissue, *viz.*, the protoderm, ground meristem and procambium, only the first can be detected at this stage. Cells of the embryonic protoderm remain highly meristematic. A few cells of the protoderm close to the suspensor exhibit "Kappe" type divisions marking the beginning of the peripheral region of the root cap (Fig. 24). In *Delphinium* two cells in the centre at the chalazal end are bigger and lightly staining than the rest of the protoderm cells (Figs. 2, 24). A few protoderm cells at the sides of these two bigger cells show periclinal divisions. In *Ranunculus* the protoderm cells at this end show predominantly anticlinal divisions. In *Nigella* periclinal divisions are observed in a few protodermal cells at the shoulders of the broader end (Fig. 10). These divisions may be indicative of cotyledonary initiation. In *Delphinium* and *Ranunculus* cotyledonary initiation occurs only later. In all the three species one or two layers of cells in the centre at the suspensor end are arranged in regular longitudinal rows forming the early columella of the root cap. A few larger and lightly stained cells in the centre towards the broader end of the embryo in *Delphinium* and *Ranunculus* mark the embryonal pith (Fig. 2). In *Delphinium* a few cells near the periphery of the columella head divide longitudinally forming thin longitudinal elongated cells. This appears to be the earliest sign in the development of procambium (Fig. 24).

Heart-shaped stage : The cotyledons appear as projections on the flanks and the embryo attains a length of about 306 μm in *Delphinium*, 300 μm in *Nigella* and 240 μm in *Ranunculus* (excluding the cotyledons). The following regions can be distinguished :

(i) A small group of cells located at the apical notch and covered by the protoderm. This group is about 2 to 3 layers deep and shows no zonation (Figs. 3, 11, 17, 25). The region is interpreted as the "Central zone of the embryonic shoot apex" by Mahlberg (1960).

(ii) At the narrower radicular pole, the organization characteristic of the mature root is already established with discrete stelar, cortical and columella initials in *Delphinium* and *Ranunculus* (Figs. 4, 18, 26) and a small group of common initials in *Nigella* (Fig. 12). In all the three species frequent "Kappe" type divisions of the protoderm contribute cells to the peripheral region of the cap. The columella is about 2 to 3 cells in width as seen in longisections.

(iii) The embryonic cortex is made up of about 3 to 6 layers of comparatively short cells. The procambial cells differentiate from the radicular end and progress acropetally towards the cotyledons. The procambial cells, initiated in the late globular stage can now be clearly discerned by the following characters, *viz.*, less frequent transverse and more frequent longitudinal divisions in cells, resulting in longitudinally elongated, narrow cells, which show relatively deep staining (Figs. 4, 18). It is significant that the procambium becomes clearly established in this stage, when early stages of cotyledonary development are also evident.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Torpedo stage : The plumular apex shows a group of cells covered by the single protoderm layer which may be referred to as the tunica. The subtunica region is about 3 to 5 cells deep and devoid of zonation (Figs. 5, 13, 20, 28). The procambial strands could be traced acropetally into the cotyledons but no trace extends to the plumular region.

In *Delphinium* and *Ranunculus* a pith made up of larger and vacuolated cells can be discerned in the centre while in *Nigella* there seems to be a solid core of procambium (Fig. 29).

The cortex had broadened. The radicular end shows a well-developed columella composed of vertical files of cells—about 10 cells deep and 5 cells wide in *Delphinium* (Fig. 6), 8 cells deep and 4 cells wide in *Nigella* (Fig. 14) and 8 cells deep and 3 to 4 cells wide in *Ranunculus* (Fig. 19). The peripheral region of the cap is solely contributed by further "Kappe" type divisions of the protoderm cells and their derivatives. The organization laid down in the heart-shaped embryo persists at the radicular pole.

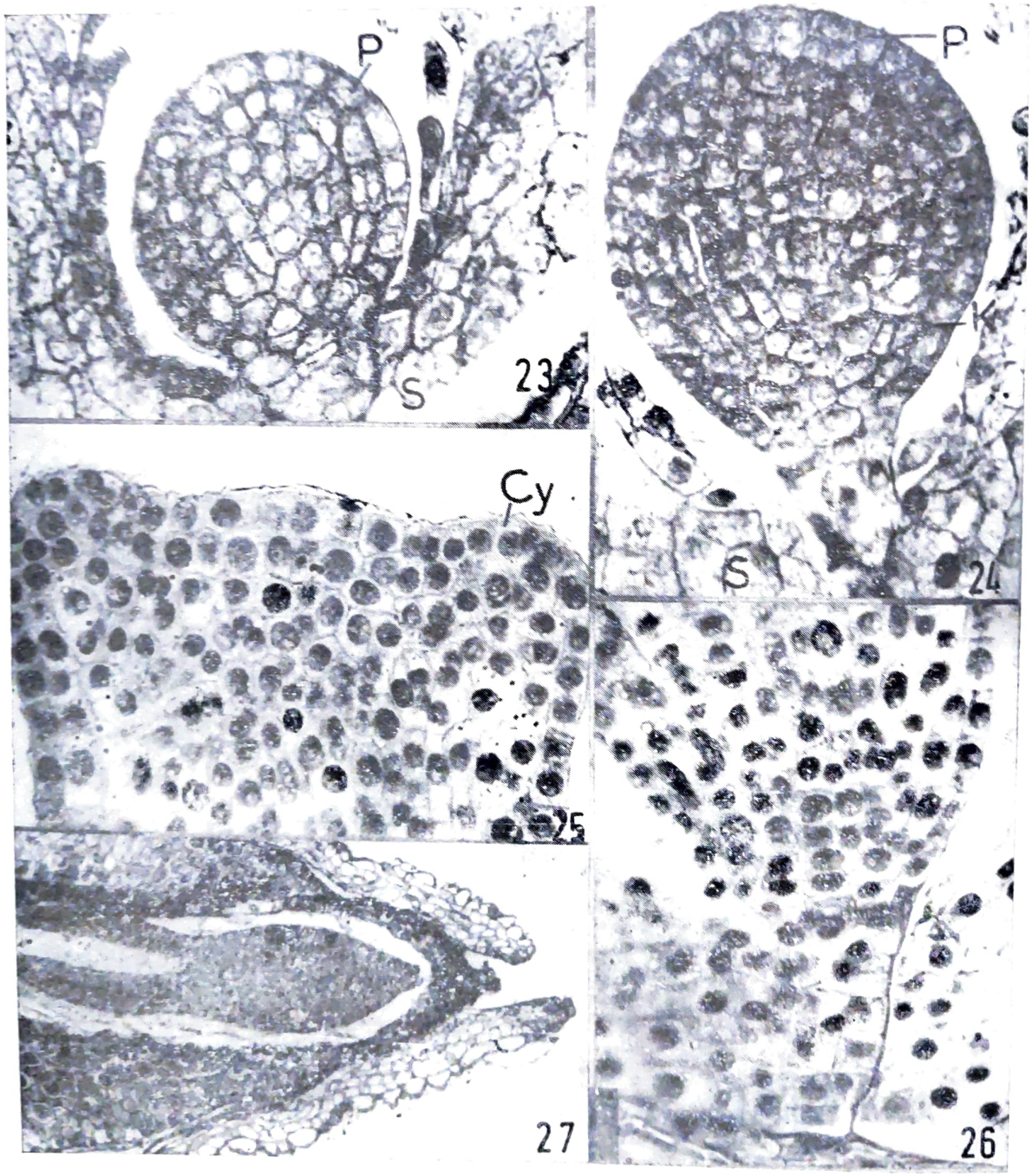
Mature stage : The embryo has grown in length and breadth. The flat plumular apex is flanked by the cotyledons and shows a single-layered tunica in all three species (Figs. 7, 15, 22, 27). The peripheral region of the cap has dwindled in size as compared to that in the torpedo-shaped embryo. The cortex is more prominent, as also the pith in *Delphinium* and *Ranunculus*. No further change in organization is evident from the previous stage except that the cotyledons have increased in length.

DISCUSSION

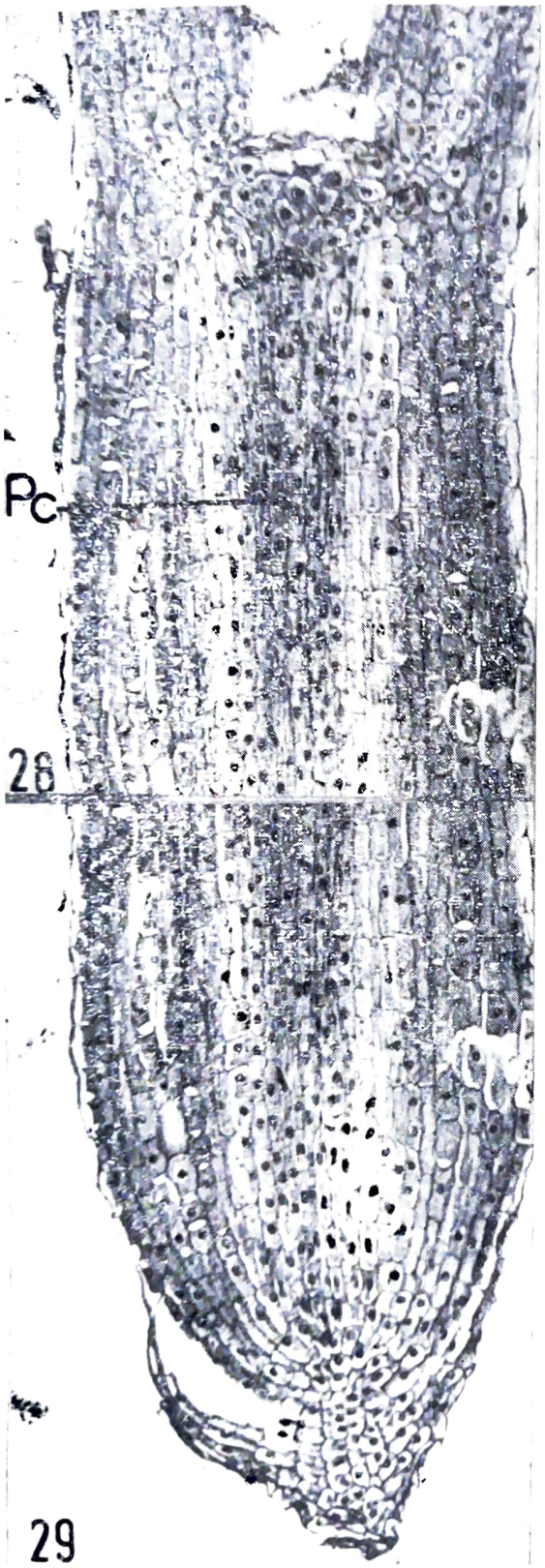
Earlier studies on gymnosperms (Schopf, 1943 ; Allen 1947a, b ; Spurr, 1949) and angiosperms (Miller and Wetmore, 1945a, Reeve, 1948 ; Guttenberg *et al.*, 1954a, b, 1955) bring out that during embryogeny the root pole differentiates first. Similar observations were also reported by Pillai and Sukumaran (1969) in *Cyamopsis tetragonoloba*, Pillai *et al.*, (1974) in *Albizia lebbek* and by Pillai and Mehra (1973) in *Tecoma stans*. On the contrary Mahlberg (1960) and Pillai *et al.*, (1975) observed that the plumular apex is the first to become distinct in the embryo of *Nerium oleander* and *Cosmos bipinnatus*. All the species reported here confirm the earlier view. Only *Nigella* shows a few periclinal divisions indicating cotyledonary initiation at the late globular stage.

Reeve (1948) raised the question about the phylogenetic significance of the earlier differentiation of the shoot pole or root pole during embryogeny. With the limited number of plants worked out here, any conclusions would not be valid, and more detailed studies of many primitive and advanced angiosperms are needed.

Figs. 1-22. Figs. 1-8. L. S. of embryos of *Delphinium ajacis*. Fig. 1 Early globular embryo. Fig. 2. Late globular embryo. Fig. 3. Chalazal end of heart-shaped embryo. Fig. 4. Suspensor end of heart-shaped embryo. Fig. 5. Chalazal end of torpedo stage embryo. Fig. 6. Suspensor end of torpedo stage embryo. Fig. 7. Plumular apex of mature embryo. Fig. 8—Radicular apex of mature embryo. Figs. 9-16. L. S. of embryos of *Nigella sativa*. Fig. 9—Early globular embryo. Fig. 10—Late globular embryo. Fig. 11—Chalazal end of heart-shaped embryo. Fig. 12—Suspensor end of heart-shaped embryo. Fig. 13—Chalazal end of torpedo stage embryo. Fig. 14—Suspensor end of torpedo stage embryo. Fig. 15—Plumular apex of mature embryo. Fig. 16—Radicular apex of mature embryo. Figs. 17-22—L. S. of embryos of *Ranunculus sceleratus*. Fig. 17—Chalazal end of heart-shaped embryo. Fig. 18—Suspensor end of heart-shaped embryo. Fig. 19 Suspensor end of torpedo stage embryo. Fig. 20 Chalazal end of torpedo stage embryo. Fig. 21—Radicular end of mature embryo. Fig. 22—Plumular end of mature embryo.



Figs. 23-27. L. S. of embryos of *Delphinium ajacis*. Fig. 23—Early globular embryo. Fig. 24 Late globular embryo. Note lightly stained bigger cells at the tip. Fig. 25—Chalazal end of heart-shaped embryo. Fig. 26 Suspensor end of heart-shaped embryo. ×500. Fig. 27—Mature embryo. ×150. (P—Protoderm ; S—suspensor ; Cy—Cotyledonary projections ; K—Kappe division).



Figs. 28 & 29. L. S. of embryos of *Nigella sativa*. Fig. 28—Chalazal end of late torpedo stage embryo. Fig. 29 Suspensor end of torpedo shaped embryo. Note a solid core of procambium (PC) in the centre (×225).

A solid core of procambium has been reported in the mature embryos of herbaceous dicotyledons like *Phlox drummondii* by Miller and Wetmore (1945) and *Pisum sativum* by Reeve (1948). On the contrary, among woody dicotyledons, the absence of a solid core of procambium in mature embryos was envisaged by Nast (1941) for *Juglans regia* and Hayat and Canright (1965) for *Annona* species. Based on this Hayat and Canright (1965) suggest that perhaps, the presence of a solid core of procambium may be a common feature of the herbaceous dicotyledons. On the contrary, Pillai and Raju (1975) did not find a solid core of procambium in *Arachis* embryo and in the present study, embryos of *Delphinium ajacis* and *Ranunculus sceleratus* also do not show a solid procambial core, whereas *Nigella sativa* does. Vijayraghavan and Marwah (1969) based on embryological studies of *Nigella damascena* do not support the segregation of Helleboreae and its elevation to the family rank. Work on Ranales by the authors has brought out that *Nigella sativa* differs from *Delphinium ajacis* and *Ranunculus sceleratus* in its shoot and root apical organization and structure of the cotyledonary node (Kavathekar 1976). The above points may add to the evidence supporting the separation of Helleboreae. Studies on other related species *vis-a-vis* *Nigella's* position in Ranunculaceae are underway.

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