STUDIES IN PTERIDOPHYTES

V. The Development, Structure and Arrangement of Leaves in Some Species of Lycopodium

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INTRODUCTION

In literature there appears to be some difference of opinion regarding the initiation of leaf in Lycopodium, whether it is initiated from a single cell or from a small group of cells. A detailed study of leaf initiation and development in all the available species of Lycopodium was undertaken to see if this can throw some light on the problem.

Out of the ten species studied here, the material of L. lucidulum Mich., L. complanatum L., L. obscurum (Ithaca, N.Y.), L. cernuum L., L. squarrosum Forst, L. setaceum Hamilt, L. clavatum L. (Nepal), and L. sp. (Ootacamund) was collected by Prof. V. Puri and passed over to me for the present study. Materials of L. serratum Thunb (Okazaki, Japan), L. clavatum var. Niponicum Nakai (Okazaki, Japan), L. nikoense French. et. Sav. (Sigakozen, Japan) were obtained through the courtesy of Dr. Y. Nozu and Dr. H. Ito; that of L. phlegmaria L. was collected at the author’s request by Mr. S. C. Verma of the Punjab University, Chandigarh, from Dikchu-Singhik Road, North Sikkim. All the species were fixed in F.A.A. or in Acetic Alcohol (1:3). Customary methods of dehydration and embedding were followed. Serial longitudinal sections and cross-sections of 6 to 15 microns of the stem and strobilus apices were cut. For staining Safranin-fastgreen Heidenhain’s hematoxylin-fastgreen combinations were used. The latter gave good result for developmental studies.

OBSERVATIONS

The leaves in all the species of Lycopodium studied here are simple, sessile, linear or lanceolate in shape having broad and sometimes decurrent...
leaf-bases. They are microphyllous and eligulate and may be light green or pale green in colour. At maturity, they may be 2–12 mm. long as in *L. complanatum*, *L. clavatum*, *L. obscurum*, *L. nikoense*, *L. setaceum* and *L. phlegmaria* or 20–30 mm. as in *L. serratum*, *L. lucidulum* and *L. squarosum* covering the stem densely. The distance between two adjacent leaves, however, increases in the trailing portion of the stem and in this region sometimes these may be completely suppressed or developed only as scaly structures as in *L. obscurum*. Two kinds of leaves have been observed in *L. complanatum* and *L. obscurum*.

*Initiation of leaf.*—Shoot apices of only mature plants of *Lycopodium* have been studied here, while a few young differentiating stem apices from bulbils have also been examined for the initial development of leaf. In all the species, the apical protuberance is protected by a rosette of acropetally developing leaves which are scaly and chaffy in *L. setaceum*, *L. cernuum* and *L. elavatum*.

The growing point shows some variation in form in different species. In *L. lucidulum*, the stem-tip is mostly flat when seen from above, and the leaf primordia initiate from the side flanks of the apex and are erect. In *L. serratum*, *L. squarosum*, *L. sp.*, etc., it may be a low mound or a completely dome-shaped structure measuring 630 μ above the youngest leaf primordium (Fig. 1). In *L. cernuum*, *L. obscurum*, *L. nikoense*, *L. clavatum* and *L. complanatum*, however, the apex is conical and projects considerably from the youngest leaf primordium (Fig. 2). This projection is more in the fertile apices than in the sterile ones. It is about 2100 μ vertically up from the youngest sporophyll in case of *L. clavatum*.

The shoot apex in all the species consists of a hump of meristematic tissue distinguishable into an outer layer covering an inner dome. Each leaf primordium is initiated more or less close to the stem-tip. A group of 4 to 5 superficial cells enlarge somewhat and then divide periclinaly (Figs. 1–4, 18, 19). Out of these two to three cells can be seen in a longitudinal section of the stem-apex. These cells are densely cytoplasmic and take a deep stain with iron hematoxylin and have conspicuous nuclei. This is followed by divisions in some underlying cells (Figs. 5, 20). A small emergence is formed which enlarges later into a small dome (Figs. 6, 21). The cells of the outer layer divide mostly anticlinally and sometimes periclinaly while those of the inner tissue have irregular divisions and thus form an elongated primordium having a dome-shaped apex (Figs. 6–10, 22, 25).
Figs. 1–17. Figs. 1 and 2. Median longitudinal sections through the growing stem-apices of *L. serratum* and *L. clavatum* 
(100 × approximately). Note the position of the initiation of leaf. Figs. 3–17. Successive developmental stages of leaf of *L. squarrosum*. Note the initiation of leaf and procambial tissue in Fig. 3 and Fig. 7 respectively (Figs. 3–15, 100 ×; 14–19, 50 × approximately).
A provascular strand differentiates very early in the ontogeny of the leaf. In a longitudinal section it can be located in second or third leaf primordium from the apex on either side. The length of the leaf primordium is only about 150 μ at this stage (Fig. 7). At first some cells in the central basal region of a leaf primordium, i.e., in the stem region become elongated. These are comparatively densely cytoplasmic and stain more deeply than the surrounding cells of the stem-apex and leaf-base. They divide rapidly in both radial and longitudinal planes but the longitudinal divisions are much more than the radial ones. With further elongation of leaf, the provascular strand starts differentiating both basipetally and acropetally (Figs. 11–17, 26, 28). The basipetal differentiation occurs quite early in ontogeny of leaf and sometimes a connection of the provascular strand of leaf primordium and that of the stem can be observed when the former is only 209 μ high from the apex. On the other hand, the acropetal differentiation of provascular strand remains in progress with the development of the leaf. In some species as in L. squarrosum, L. clavatum and L. sp., etc., the procambial tissue has not been observed up to the tip of the leaf.

**Further development of leaf.**—After the formation of the procambial strand the leaf primordium shows marked increase in its length (Figs. 10–17, 25–28). All the cells remain active in L. squarrosum and L. sp. up to the time the leaf primordium attains a length of 950 μ. All of them possess prominent nuclei which take a deep stain and divide mostly transversely thus contributing to the length of the leaf. With further elongation the leaf-tip becomes somewhat conical in shape in a longitudinal section and its terminal cells begin to show sign of maturation as sudden enlargement in the outline of cells, development of vacuolar system and absence of division figures, etc., are noted. Maturation of apical cells in the leaf-tip of L. squarrosum is observed when the primordium is only 1305 μ in its length. The cells below these terminal cells remain active. Division figures have been observed in the outer layer as well as in the inner tissue, transverse divisions at this stage being more common than longitudinal ones. When the leaf attains a length of about 1451 μ the basal cells also show sign of maturation while the cells which are in between the apex and the base of the leaf are meristematic in nature. The growth in the leaf of the Lycopodium is, therefore, intercalary for a considerable period. Such intercalary growth in case of L. squarrosum has been observed when the leaf becomes 1828 μ in length. Finally, a considerable increase in the leaf tissue is due to elongation and enlargement of individual cells.

Early stages of development of a sporophyll are the same as those of a sterile leaf. The sporangium develops very early in the ontogeny of the
FIGS. 18-28. Successive developmental stages of the initiation and early development of leaf. Figure 18 shows periclinal divisions in the outer layer of the stem-apex, which are responsible for the initiation of leaf (500 × approximately).

leaf. The first sign of its initiation is periclinal divisions in a group of adaxial epidermal cells of the leaf-base. Out of this group only 3 to 5 peri-
clinally dividing cells can be observed in a vertical longitudinal section (Fig. 29). The outer cells formed by such divisions form the wall which becomes multi-layered while the inner ones give rise to the sporogenous tissue (Figs. 30–33). The innermost layer of the sporangial wall becomes the tapetal layer (Figs. 34–37). With the development of sporangia in fertile leaves the cells which are present just below the sporangium on the abaxial side also show signs of maturation. Further growth is intercalary in sporophylls for a considerable period as in sterile leaves.

Arrangement and anatomy of leaf.—The arrangement of leaf is usually spiral; however, it varies in different species, within the same species and even sometime in the same plant on different branches. In _L. complanatum_, _L. phlegmaria_ it is 2/5; in _L. obscurum_, _L. nikoense_ 2/7 and in _L. squarrosum_ 2/13. In _L. clavatum_ it may be 2/9, 2/11 and in _L. lucidulum_ and _L. serratum_ it may be 2/8, 2/9, 2/10 and 2/11 (Figs. 40–44). In mature plants the phyllotaxis appears to be opposite in _L. complanatum_ and whorled in _L. nikoense_ (Figs. 38, 39).

A cross-section of mature leaf shows a well-developed cuticularized epidermis having stomata either on one surface or on both surfaces (Fig. 45). The mesophyll is composed of the isodiamatric cells having intercellular spaces. Sometimes inner tissue may be of thick-walled cells. The vascular bundle is mesarch and in some leaves never reaches up to the tip. It may be feebly (_L. squarrosum_, _L. serratum_, etc.) well-developed (_L. complanatum_, _L. nikoense_, etc.) (Figs. 45, 46).

The mature leaves of _Lycopodium_ are usually dorsiventral (_e.g._, in _L. serratum_, _L. obscurum_, _L. lucidulum_, _L. squarrosum_, etc._) or they may be tetragonal in cross-section as in _L. nikoense_ and scaly like those of _Thuja_ as in _L. complanatum_. It is interesting to point out that dorsiventral leaves of different species are tetragonal in appearance in their juvenile stage.

The structure of mature stomata and their mechanism of closing and opening in different species is well known (Copeland, 1902 and Chowdhury, 1937); but we know little about their development. The stomatal mother cell in _L. squarrosum_ can be easily recognised owing to its deep staining capacity and dense cytoplasm. It is brick-shaped in cross-section and has a prominent nucleus. When examined in sufrace view its enlargement results in acquiring an oval shape. The nucleus of the stomatal mother cell then undergoes mitosis by forming a transversely placed spindle. A longitudinal or anticlinal wall is then formed giving rise to two longitudinally elongated
FIGS. 29–52. Fig. 29. Portion of a vertical longitudinal section of a young sporophyll of L. clavatum showing the rudiment of the sporangium (500 x approximately). Figs. 30–33. The same in advanced stages (500 x approximately). Fig. 34. Portion of the young sporangial wall of L. squarrosum from a vertical longitudinal section (250 x approximately). Fig. 35. The same in advanced stage (250 x approximately). Figs. 36 and 37. Vertical longitudinal sections of the mature sporangium of L. squarrosum and L. clavatum respectively (60 x approximately). Figs. 38–40. Transverse sections just below the stem-apex of L. obscurum, L. complanatum and L. nikoense respectively showing the arrangement of leaves (100 x approximately). Figs. 41–43. The same in three different branches of L. serratum (24 x approximately). Fig. 44. The same in L. squarrosum (24 x approximately). Fig. 45. Vertical section of the leaf of L. squarrosum (150 x approximately). Fig. 46. Transverse section of the vascular strand of leaf of L. nikoense (1000 x approximately). Figs. 47–51. Development of stomata in L. squarrosum (500 x approximately). Note the elongation of stomatal mother cell in Fig. 48 and metaphase condition in Fig. 49. Freshly formed guard cells and a young stoma with pore in Fig. 50. Fig. 52. Stomata and epidermal cells of a leaf of L. squarrosum in surface (50 x approximately).

( cut., Cuticle; ep., epidermis; gu.c., guard cell; i.l., initiating leaf; i.sp., initiating sporangium; i.t.t., inner tissue; l.t., leaf trace; me., mesophyll; mu.ca., mucilage cavity; o.l., outer layer; ph., phloem; pro. ti., provascular tissue; sp., sporangium; sp.t., trace of the sporophyll; st.m.c., stomatal mother cell; ta., tapetum; xy., xylem.)
guard cells which continue to enlarge for some time and develop later on a lenticular pore in between. The adjacent epidermal cells which surround the guard cells undergo some adjustment in their shape to accommodate the two newly formed guard cells (Figs. 47–52). Just after the division of the stomatal mother cell an intercellular space begins to develop by their side in the tissue of the leaf. It also increases in its dimensions with the formation and enlargement of guard cells. The average dimensions of each guard cell is $65.3 \times 17.5 \mu$, aperture is $21.1 \times 7.7 \mu$ while the epidermal cells are $153.5 \times 32.7 \mu$ in \textit{L. squarrosum}.

The present study of stomatal development, therefore, confirms that the stomata in \textit{Lycopodium} is of haplocheilic type and the neighbouring cells of stomata are pergenous in origin (Figs. 47–52).

\section*{Discussion}

Since there is some controversy regarding the initiation of leaf in \textit{Lycopodium}, it may be worthwhile to focus some attention on this aspect in the light of present observations.

The first point is the initiation of leaf whether it is by a single cell or by a small group of cells. It was Turner (1924) who traced a single cell in \textit{L. lucidulum} for initiation of leaf. Wigglesworth (1907) was of the opinion that the formation of leaf in \textit{L. clavatum} and \textit{L. complanatum} is by the activity of a few cells and postulated that an apical cell later on becomes evident for some time in the leaf-tip. Campbell (1913, 1940) held a similar opinion. While making a study of apical organisation of shoot in some species of \textit{Lycopodium}, Hartel (1938) pointed out that a leaf primordium initiates from the outer several layers of cells near the stem-apex. It is, however, pertinent to note that she made a casual study of the leaf initiation as she stressed mainly on the apical organization of stem.

As already recorded the leaf in \textit{L. clavatum}, \textit{L. squarrosum}, \textit{L. complanatum}, \textit{L. serratum}, \textit{L. lucidulum} and \textit{L. phlegmaria}, makes its appearance by the activity of some superficial cells which simultaneously become associated with some of the inner tissue present below the outer layer of the apex. No evidence could be found for the presence of a single apical cell as pointed out by Turner (1924) or an apical cell which becomes evident after the initiation for further development of leaf primordium as postulated by Wigglesworth (1907).

In other genera of the lycopods the consensus of opinion is that the leaf arises from a group of superficial cells rather than from a single cell. Treub
(1876) and Bruchmann (1897), both quoted from Sifton (1944), for instance, described the formation of leaf in *Selaginella* by the activity of a few cells on the lateral flanks of the apex and postulated that an apical cell later on becomes evident for some time in the leaf-tip. In *Isoetes* Farmer (1890), Smith (1900), West and Takeda (1915) and Bhambie (1963) reported that the leaf rudiment develops as a result of luxuriant growth of some superficial cell although Stewart (1948) pointed out that leaf primordium in *Isoetes* owes its origin to the activity of an apical cell. This observation appears to be rather casual as Stewart has mainly dealt with plastids of *Isoetes*. In *Phylloglossum* Campbell (1913) believed that none of the organs shows a single apical cell.

In true ferns although it was believed previously that a single cell is responsible for the initiation of leaf (Hofmeister, 1868; Debary, 1884; Bower, 1923; Campbell, 1940; Sifton, 1944; Wardlaw, 1945). Wardlaw (1949, 1952) mainly working on the morphogenesis of shoot apices of certain ferns, *e.g.*, *Dryopteris aristata*, etc., arrived at the conclusions that a single apical cell seldom gives rise to the leaf initiation; rather it is from a group of superficial cell. A single cell, however, becomes prominent very early in the primordium of the leaf.

After the initiation of the leaf in *Lycopodium*, the primordium grows by the activity of all the cells till the leaf attains considerable size. Later on the terminal cells as well as the basal cells of the leaf show sign of maturation. Further growth of leaf is intercalary followed by cell enlargement.

The development of stomata is of the haplocheilic type. Florin (1951) is of the opinion that haplocheilic type is more primitive than the syndetocheilic type. Besides *Lycopodium* haplocheilic type of stomata has also been recorded for other fern allies, *e.g.*, *Isoetes* (Bhambie, 1963) and *Psilotum* (Pant and Mehra, 1963).

**Summary**

This paper describes the ontogeny and structure of leaf in some species of *Lycopodium*. The leaf originates from a group of a few superficial cells which later on become associated with some cells of the inner tissue of the stem which is just beneath the outer layer of the stem apex. To begin with every cell of this primordium is meristematic. Later on, sign of maturation of cells becomes evident in the apical-most cells and the basal cells of the leaf and the further growth thus becomes intercalary. Lastly, a considerable increase in the leaf tissue is due to elongation of individual cells. The anatomy of leaf is simple. It is traversed by a single mesarch vascular bundle. The
arrangement of leaf is, however, quite variable in different species of *Lycopodium* as well as in the same species and even in the same plant at different regions. The stomata is of the haplocheilic type.

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