

A STUDY ON THE ROOT OF OPHIOGLOSSUM PETIOLATUM HOOK.⁽¹⁾

YU-SHIAW CHEN⁽¹⁾ and YOU-LONG CHIANG⁽²⁾

Abstract: Growth habit and both internal and external structure of root of *Ophioglossum petiolatum* Hook. were investigated. It was found that the sporophyte of this plant possesses many characteristics which are highly adapted to a dry terrestrial habitat. Although the roots are without root hairs, the internal structure of the root shows that the smooth surface of root epidermis is the site of water absorption. The Casparian strip is not continuous, but rather is broken by many minute pores. This observation leads us to suggest that these openings function in promoting the periclinal translocation of sugar from the sieve cells to every endodermal cells, so that the endodermis may proceed smoothly in its osmotic absorption of water into the stele.

INTRODUCTION

Ophioglossum petiolatum Hook. is an eusporangiate fern. There are about 25 recognized species of *Ophioglossum* (Clausen, 1938, Smith, 1955). Four are known from Taiwan (DeVol, unpublished, 1968). Ophioglossales has been treated by Cronquist (1961) as an order in a single class Filices. It is a small order of only four genera which are all living. All other orders of eusporangiates are only known as fossils, except the Marattiales which has both living and fossil species. Ophioglossales are modern ferns with apparently primitive characters. The studies on this genus which have been done since the end of the last century are by Boodlee (1899), Bower (1896, 1904), Chrysler (1910, 1941), Petry (1914), Clausen (1938), Joshi (1940), Nishida (1952), Kurita and Nishida (1965), Nozu (1955, 1956), Peterson (1967, 1969a, 1969b, 1969c) and Bienfait (1969).

MATERIALS AND METHODS

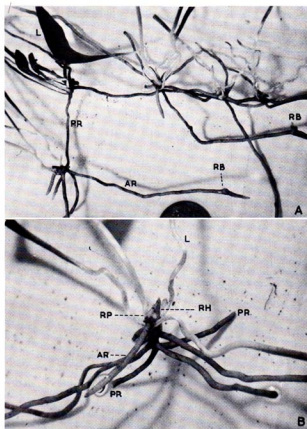
Plant materials were collected from the campus of Fu-Jen Catholic University at Hsin Chuang, Taiwan in late July, 1970. Some were harvested on the campus of National Taiwan University, Taipei, in early November. Still other materials were found in a flower pot in which a *Begonia* plant was planted. They were identified as *Ophioglossum petiolatum*. Plants were harvested and planted in the flowerpots (10 to 15 cm in diameter) filled with garden soil. They were cultivated in the green house of our Botany Department. The flower pots were kept moist by setting them in water in a shallow trough.

In order to prevent mechanical damage while collecting the roots for investigation, the soil was washed away from the roots in running tap water. The roots

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(2) Assistant in Botany, National Taiwan University, Taipei. This paper was based upon a thesis submitted by her in partial fulfillment of her M.S. in botany. 陳玉霞

(3) Professor of Botany, National Taiwan University, Taipei 江有龍



Figs. 1A-1B. Photographs showing the external morphology of adventitious root of *Ophioglossum petiolatum*. A, a photograph showing that the adventitious root (AR) connects several plants together to form a whole plant. Two root-buds (RB) at different ages are seen in this figure, $\times 1$. B, a photograph showing the growth of adventitious roots and leaves from a rhizome, $\times 2$. PR, parent root; RP, root primordium.

were fixed in FAA immediately after harvesting. After dehydration with a tertiary butanol series, the materials were embedded in the paraffin blocks. Serial sections were made at a thickness of 8μ and stained with Safanin O and fast green (Johansen, 1940). Root tips were specially stained with orange G, tannic acid, and iron alum according to the schedule of Sharman (1943).

In the study of tracheary elements, the materials were cut into pieces of 1-1.5 cm long, and macerated in a solution containing 1 part superoxal, 4 parts distilled water and 5 parts glacial acetic acid in oven for 3-4 days. After washing in distilled water several times, the materials were dehydrated with ethyl alcohol, stained with safranin and mounted in Canada balsam.

RESULTS

Growth habit and external morphology.

Ophioglossum petiolatum is about 10 cm high (Fig. 2). It is weedlike and often found growing among various weeds on a dry, open campus. It however grows equally as well in water saturated soil. *Ophioglossum* is world-wide in distribution (Simth, 1955). (It was observed that *O. petiolatum* is not a "mass" plant, i.e., not a plant which can be collected from everywhere in large quantities.) When this plant is found, several plants are nearly always found growing together. They are connected to each other by horizontal roots (Figs. 1A, 2). It is seldom found growing singly. Many observations show that this plant reproduces vegetatively mainly by root buds borne on the horizontal roots rather than by the production of gametophytes. The roots of *O. petiolatum* are very shallow, growing about 3-4 cm beneath the surface of soil (Fig. 2). Its small size and its association with weeds help to reduce the excess loss of water vapor. It is adapted to a dry terrestrial habitat, and so is known as a terrestrial herb (Foster and Gifford, 1958).

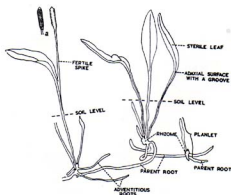
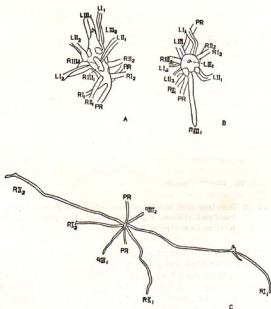


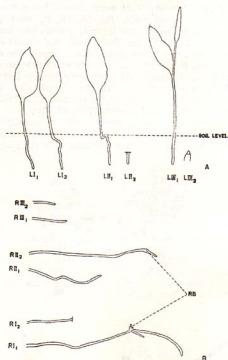
Fig. 2. A drawing showing the growth habit of *O. petiolatum* cultivated in a flower pot, $\times 1$. a, the adaxial surface of sporangial area.

Each individual plant has one short erect rhizome (about 3 mm long) which bears one to several leaves and roots. (Fig. 1A, 1B, 2). Most species of Ophioglossales growing in the temperate zone typically produce only one leaf each year (Smith, 1955; Cronquist, 1961). In the green house, we observed one individual rhizome which produced 4 to 5 leaves in a growing season. Since the rhizome is very short, it is difficult to recognize the order in which new leaves and roots are produced on the rhizome. In general, the successively younger leaves and roots are produced successively on the upper portion of the rhizome (Figs. 3A, 3B). A close correlation was observed between the number of leaves and of roots, e.g., a plant with 5 leaves always has 5 adventitious roots (Figs. 4A, 4B).

A single rhizome may give rise to several roots horizontally in all directions (Figs. 3A, 3B, 3C). Each root produces a root bud or adventitious bud on the upper side of the root at about 8 mm behind the root apex when the root grows to about



Figs. 3A-3C. A and B, drawings of a rhizome showing the attachment of leaves and roots, $\times 3$. B is a top view. L, leaf; R, root; PR, parent root. The Roman numerals I, II, III indicate the root pair or leaf pair which are successively younger. The Arabic number 1 indicates the younger root or leaf of a pair, e.g., R₁ indicates the first or younger root of the first or the oldest root pair. L_{II} and R₁ are unfortunately lost. C, a drawing showing the growth directions of roots, $\times 1$. Note their regular arrangement in pairs.



Figs. 4A-4B. Drawings of all leaves and roots which are given rise from an individual rhizome, $\times 1$. RB, root bud. This plant is the same plant as illustrated in Figs. 3A-3C.

8-10 cm long (Fig. 3C, 4B, 5). Every horizontal root is capable of indefinite growth, giving rise to new individuals which are connected each other. Since the connecting root or parent root persists for a long time, *Ophioglossum petiolatum* may be considered as a complex organism, i.e., it is an aggregation of individuals linked together by subterranean horizontal roots. This type of growth habit is somewhat similar to *Marsilea* and the psilophytes although the horizontal connecting organ of the later are stolons or rhizomes and not a root.

All roots of *O. petiolatum* are adventitious roots, and are fleshy and cylindrical. Since the roots are devoid of secondary growth, they vary little in thickness, being about 1-1.5 mm in diameter. Root hairs are absent. The root is brown excepting at the tip which is white. It is incapable of branching, even if the root tip is cut off or mechanically damaged. As shown in Fig. 3C, the roots occur in opposite pairs, i.e., the 1st root is opposite to the 2nd, 3rd to 4th, and 5th to 6th (Figs. 3A-3C, 4A-

4B). Leaves are also found occurring in pairs, *i. e.*, the 1st leaf is opposite to 2nd leaf, 3rd to 4th and etc. (Fig. 3A, 3B, 4A).



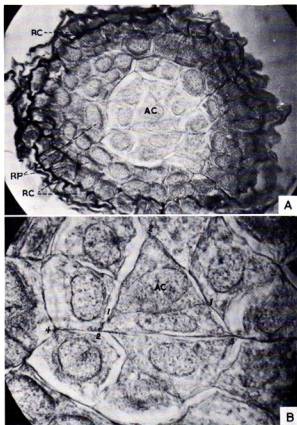
Fig. 5. Drawings indicating the successive stages of the development of root bud, $\times 1$. Note that root buds occur about the same distance from rhizome.

Internal structure of root.

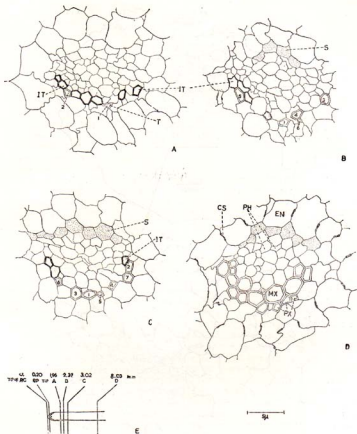
The root of *O. petiolatum* consists of root proper and rootcap (Fig. 6A). The latter is a cup-shaped structure in which the tip of the root proper is closely covered. The walls especially the periclinal walls of the cap cells are thicker than those of meristematic cells which are enclosed in the cap (Fig. 6A). No intercellular spaces are present in the root cap. The rootcap is about 100μ to 300μ thick. The length of the cap is about 600μ or more. The root apical cell of this plant is tetrahedral or pyramidal with four dividing planes. Its basal wall is watch-glass-shaped, facing its convex face toward the rootcap. The height of the pyramid is 40μ . Rootcap tissue arises from the basal face of the apical cell. The three side faces of the apical cell are approximately triangular and more or less flat. All tissues of root proper arise by regular divisions from these lateral faces in cyclic order (Fig. 6B).

The cortex is the earliest tissue distinguishable from the procambium and is recognizable by its position and cell size. The cortical cells are larger, whereas the cells of central procambium are smaller. The differentiation of xylem in the procambium begins with the formation of tracheids which appear in the ventral side of the procambium (Figs. 7A, 9A). In one root, we observed that the first tracheid appeared at 1.38 mm behind the apex of the root proper (Fig. 7A, Fig. 9A). The second tracheid appears at its left side, *i. e.*, 3 cells apart from the first one. These two tracheids, together with seven other developing tracheids constitute a discontinuous xylem arc. The number of tracheids continues to increase as the root grows older. As in Fig. 7C, the number of mature tracheids increased to seven in a section cut at about 3 mm behind the root tip. As shown in Figs. 7B and 7C; although the sequence of the maturation of tracheids is irregular, the next tracheid to be matured is always found some distance from the tracheid which has just matured.

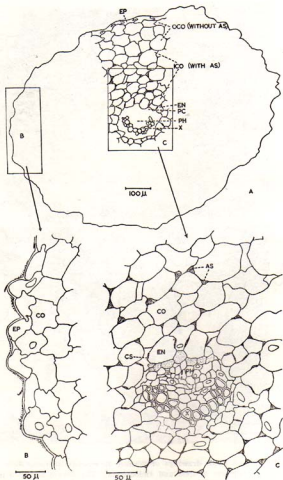
Following the differentiation of tracheids, several sieve cells appear opposite to the xylem arc. They are distinguishable because their walls are polyangled and slightly thickened, also because they are clear, being without cytoplasm and nuclei. They are somewhat larger than the other undifferentiated parenchyma cells in the phloem. Four sieve cells are first found occurring in a section 2.37 mm behind the tip of the root (Fig. 7B). They are arranged in an uniseriate layer adjoined to the undifferentiated endodermis. The sieve cells are 7 in number in a section at 3.0 mm behind the tip of the root (Fig. 7C). In this section the xylem arc is still discontinuous. The Casparian strip appears 3.8 mm behind the root tip. In a



Figs. 6A-6B. Two photographs showing transverse section of root tip through a single apical cell. Arabic numerals indicate the sequence of apical segmentation. AC, apical cell; RC, root cap; RP, root preper; A, $\times 500$. B, a photograph which is magnified from Fig. 6A, $\times 1240$.



Figs. 7A-7E. Camera lucida drawings of transverse sections of root, showing the development of stele and endodermis. The Arabic numerals in each drawing indicate the sequences of appearance of tracheids, i. e., 1 indicates the first tracheid. Note that number 2 is absent in 7B because the second tracheid in 7A is too short. Dotted cells are sieve cells (S); CS, Casparian strip; EN, endodermis; IT, immature tracheid; MX, metaxylem; PH, parenchyma; PX, protoxylem. Fig. 7E, indicating the positions where each section were cut, $\times 4$.



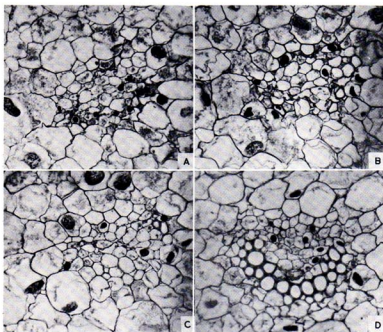
Figs. 8A-8C. A transverse section cut at about 2 cm back from the root tip. A, $\times 130$; B, C, $\times 330$. Various tissues seen in this region of the root are mature except that several cells around the tracheids are still immature. AS, air space; CO, cortex; CS, Casparian strip; EN, endodermis; EP, epidermis; ICO, inner cortex; OCO, outer cortex; PC, pericycle; PH, phloem; X, xylem; The dotted cells in the stele are sieve cells.

section cut 8.03 mm behind the root tip, we saw a mature stele with a continuous xylem arc (Fig. 7D).

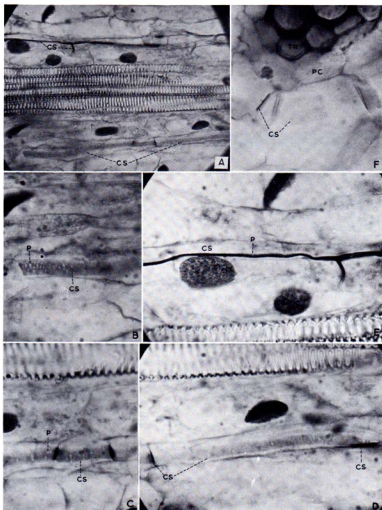
The mature root is differentiated into epidermis, cortex and stele (Fig. 8A). The transverse section is nearly round, but sometimes appears more or less flattened (Fig. 8A). The outermost layer of the root is the epidermis. Like all other species of *Ophioglossum* it has no root hairs. Its outer wall is considerably thickened and stains blue-violet with safranin O, and fast green. If we immerse a living root into water and then pick it up, the root surface will be thoroughly wetted. This indicates that the root surface is hydrophilic, *i. e.*, it has a spongy-like layer functioning in water absorption. It, however, has previously been stated (Smith, 1955; Nozu, 1956) that this thickened outer surface of the epidermis is suberized. The cortex consists of two concentric regions. The outer cortex is about 100 μ in thickness and is composed of about three layers of cells without intercellular spaces. The inner cortex is about 150 μ thick, consisting of somewhat smaller cells in about five cell layers with numerous intercellular spaces. The cortex occupies a major part of the root tissue approximately 98%. Both the inner and outer cortex is composed of a spongy-like parenchyma tissue, containing numerous starch grains. Evidently it is a storage tissue for food and water. According to Smith (1955), this layer contains a mycorrhizal fungus. But we failed to observe it in our materials which were relatively young. Perhaps the fungus is present only in older roots. The stele is much like other protosteles, being surrounded by an endodermis (Figs. 8A, 8C). The endodermis is an uniseriate sheath of cells, without intercellular spaces. In cross section it appears to form a ring consisting of about 20 cells. The cells are smaller than the cortical cells. The central portion of the radial walls of the endodermal cells has Casparian strips. Under an oil immersion lens, we observed many small openings (pores) of various sizes (0.4–1.0 μ in diameter), passing through the Casparian band which connects the cell contents of two adjacent endodermal cells. (Figs. 10A–10F). This finding leads us to suggest that these openings function in promoting the translocation of sugar from the sieve cells which are located only on the dorsal side of the stele to every endodermal cell.

Although the horizontal root is cylindrical, its internal structure shows dorsiventrality. On the dorsal side of the stele adjoining the endodermis, there is an uniseriate layer of sieve cells (Fig. 8C), these are somewhat irregular as seen in transverse section. The shorter sieve cells are 750–830 μ whereas the longer cells are above 1,000 μ in length. Boodle (1899) believed that these cells were sieve tubes. According to Esau (1961), a sieve tube is a series of sieve cells connected one after another at the sieve plate. We, however, failed to find any sieve plates in these cells and confirm them as sieve cells instead of sieve tubes. It seems to the present authors that the tapering sieve cell is more primitive than sieve tube.

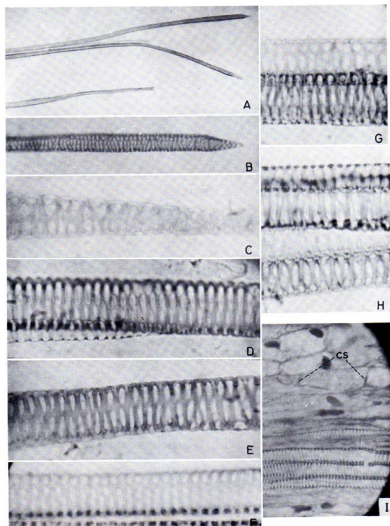
Between the sieve cells and xylem there is phloem parenchyma. In one section examined, the xylem was composed of 23 tracheids surrounded by a layer of parenchyma cells (Figs. 8C, 9D). Protoxylem and metaxylem are distinguishable in a mature xylem. The tracheid cell is nearly round and polyangled in transverse section. It is a long and slender cylindrical cell of about 4–5 mm, with tapering ends. (Figs. 11A–11I). It is scalariform, with many oval openings (pits) arranged regularly in 4 to 5 vertical rows. On the ventral side of the stele, there are one or two layers of parenchyma cells between the xylem arc and the endodermis. This layer(s) extends on the dorsal side of the stele along the endodermis, meets and joins the edges of the sieve cell layer, forming a ring. Ontogenetically it is suggestive of a



Figs. 9A-9D. Microphotographs showing the development of central stele. A-C are the photographs of Figs. 7A-7C, $\times 1240$. D is the photograph of Fig. 8C, $\times 1200$.



Figs. 10A-10F. Photographs showing the structure of Casparian strip. CS, Casparian strip; P, pore on Casparian strip; PC, pericycle; TR, tracheid. A, $\times 300$; B-E, $\times 850$; F, $\times 1050$.



Figs. 11A-11I. Photographs showing the structure of tracheids. A, $\times 85$, B, $\times 350$:
C-H, $\times 1050$.

pericycle.

DISCUSSION

In the present study, it has become clear that the sporophyte of this fern possesses many characters which are highly adapted to a dry terrestrial habitat. This is probably the most important reason why this primitive fern is still in existence while its many other relatives are now extinct.

Nozu (1956) studied the root of 5 species of *Ophioglossum* (he did not include *O. petiolatum*), among which he noted that only *O. vulgatum* is able to produce branch roots. *O. petiolatum* which we have studied does not produce branching roots.

The internal structure of the root of this fern is bilaterally symmetrical as seen in cross section. As noted earlier, the phloem strand occupies the dorsal side whereas the xylem strand occupies the ventral side of the stele. In the veins of the dorsiventral leaves of higher plants, the phloem is usually on the dorsal (lower) side and the xylem on the upper. It is currently believed that a symmetrical flower is more primitive than an asymmetric flower. Similarly, the authors of the present paper think that the dorsiventral root of *O. petiolatum* with bilaterally symmetry is more advance than roots with radial symmetry.

The root of *O. petiolatum* looks like a stolon rather than a root, because it grows horizontally and produces vegetative buds. Further it has no root hairs on its surface but is covered by a cuticle-like layer. It has a hydrophilic layer on its epidermal surface, which permits water to enter freely through the epidermis into the cortex; and has a typical Casparian strip which functions in osmotic water absorption; it also has well-developed tracheids arranged in an arc layer facing the endodermis, facilitating the entrance of water, i.e., water travels only a short distance from endodermis into tracheids.

Prantl and Poirault (cited in Boodle, 1899) who studied the stelar anatomy of *Ophioglossum*, state that most species have a monarch stele excepting *O. pendulum* and *O. palmatum*. Boodle (1899) found the monarch structure at the base of diarch root in *O. pendulum* and said that the monarch structure is probably formed by reduction of the diarch structure. Boodle did a lot of research on *Ophioglossum* but did not study *O. petiolatum*. We have found that the first several tracheids of *O. petiolatum* occur in an irregular sequence. And because of the absence of two definite xylem groups, it is impossible to considered it as a diarch stele. Therefore we confirm it as a monarch structure like most other species in *Ophioglossum*.

Recently, it has become clear that the formation of xylem and phloem is related to the content of auxin and sugar (cited in Adams *et al.*, 1970). The phloem tissue gets larger when the sugar content increases and the xylem tissue becomes bigger if the sugar content decreases. In the root of *O. petiolatum*, the phloem tissue is always on the dorsal (lower) side and the xylem tissue is on the ventral side. This differentiation of dorsiventrality may be related to the content of sugar and auxin in the undifferentiated stele. This idea needs further experimentation.

The Casparian strip, with the existence of pores on it, is a new discovery. Up to now, it has never been mentioned in literature. The writers suggest that the small openings on the Casparian strip may be related to the lateral translocation of water among the neighbor endodermal cells; besides, they function in promoting the periclinal translocation of sugar from the sieve cells to every endodermal cells,

so that the endodermis may proceed smoothly in its osmotic absorption of water into stele. If the small pores are absent, the water absorption of the root would be much slower, and the adaptability of this plant to dryness might be well affected, too. Whether or not the phenomenon of pores on the Casparian strip only occurs in this species is still unknown.

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