VASCULAR ELEMENTS IN THE CORM OF ISOETES TAIWANENSIS[1]

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Abstract: Primary and secondary vascular tissues of the corm, and the vascular tissue in the leaf and root traces of the new species *Isoetes taiwanensis* DeVol are investigated. In the secondary vascular tissue three kinds of cells are observed i.e. parenchyma, tracheids, and sieve cells. The parenchyma cells are nucleated and filled with cytoplasm; the sieve cells have several sieve areas on their walls except on their tangential walls; the tracheids have one to several-bands of lignified secondary thickening as seen in sectional view.

INTRODUCTION

The literature on the anatomy of *Isoetes* goes back to 1845 to a paper by Von Mohl, since that time the question of the nature of its cambium activity has afforded a fertile field for discussion.

It has past through the examinations of Hofmeister (1862), Russow (1872), Farmer (1890), Scott & Hill (1900), Smith (1900), Stokey (1909), West & Takeda (1915), Paolillo (1963), and Esau (1969). The interpretation of the cells in the secondary vascular tissue of *Isoetes* has swung back and forth between that of sieve elements and parenchyma cells and to even tracheids. This situation may be due to differences of interpretation, or there is the possibility that the tissues vary in different species or even in different specimens of the same species. In view of the lack of harmony in interpretation, it has seemed advisable to make an investigation regarding the anatomy of this new species which has been recently discovered in Taiwan (DeVol, 1972).

MATERIALS AND METHODS

The materials used in the present investigation were collected in a shallow pond on Seven Star Mountain, Chih-hsin-shan (七星山).

The materials were fixed in FAA (Johansen, 1940) immediately after collection. After being washed in 50% ethanol, the specimens were dehydrated through a tertiary-butanol series and embedded in paraffin. Serial transverse and longitudinal sections were cut at the thickness of 8μ–10μ and stained with either safranin and fast green (Jensen, 1962), or by tannic acid and iron alum with safranin and orange G (Sharman, 1943).

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RESULTS

Primary vascular tissue: The primary vascular tissue is composed of a lacunated central xylem cylinder surrounded by a phloic mantle (Fig. 1A). The central xylem cylinder is solid in the regions near both the apical and basal meristems, but lacunated towards the center. It consists of parenchyma cells and tracheids. The parenchyma cells are irregular, nucleated and filled with cytoplasm. The tracheids in the solid tip are short, irregular but somewhat elongated with their long axes parallel to the corm axis except near the leaf traces. Its lignified annular or spiral thickenings are distinct and neither cytoplasm nor nuclei are present. The shape of the tracheids are different from those in the leaf-and root-traces which are longer and tubular. When the central xylem becomes lacunated, most of the parenchyma cells are still connected with each other and make a reticulated bridge. Tracheids become more stretched and some of the segments of the secondary thickenings disperse throughout the lacuna.

The phloic mantle is composed of parenchyma cells and sieve cells. The parenchyma cells are the same as those in the xylem. The sieve cells are short, irregular in shape and have irregularly reticulated, thickened primary walls. The sieve area is inconspicuous. The phloic mantle is often interrupted by the passing leaf traces. The xylem of the leaf traces is continuous with the central xylem cylinder, whereas the phloem is connected with phloic mantle (Fig. 1B). It is difficult to distinguish the cells of the phloic mantle from the derivatives of the cambium because they are so compact. The cambium always begins to divide before the maturation of the tracheary elements in the central xylem cylinder, so the primary phloem is mainly recognized by its location and its cell centents.

The cambium: The cambial cells and their close derivatives are flat in radial and transverse views. In tangential view, they are pentagonal or hexagonal. They are compactly arranged and identical in both shape and contents. The cambial zone consists of two to three cells. The cambium gives rise externally to the paranchyma cells which take part in the formation of the secondary cortex, and gives rise internally to tracheids, sieve cells and parenchyma cells which form the secondary vascular tissue.

Secondary vascular tissue: The three kinds of cells which arise from the cambium are similar in outline. They are prismatic in shape and are flat in both radial and transverse views, but pentagonal or hexagonal in tangential view (Fig. 4.). The presence of the nuclei and the dense cytoplasm in the parenchyma cells make it easy to separate these cells from the tracheids and sieve cells,

The sieve areas of the sieve cells are relatively large and are often crowded so that the thicker walled parts display a scalariform or reticulate pattern (Figs. 2C, 3A, 3C, 4A). The sieve areas are always found on the radial and transverse walls but not the tangential walls (Figs. 3C, 4A). From the tangential section, the thicker parts on radial and transverse walls appear in a bead-like pattern (Figs. 3C, 4A). The kind of thickening is similar to the walls of parenchyma cells when stained with either tannic acid-iron alum or safranin-fast green combination. But it is denser in the sieve cells, and no lignin is observed.

The tracheids are different from tracheids in other vascular plants. They are cubical to prismatic, some of them are short rod-like in shape (the transverse wall is more or less isodiametric). The morphology of the secondary wall in these tracheids is variable. Most of them have one-band of secondary thickening (Figs.

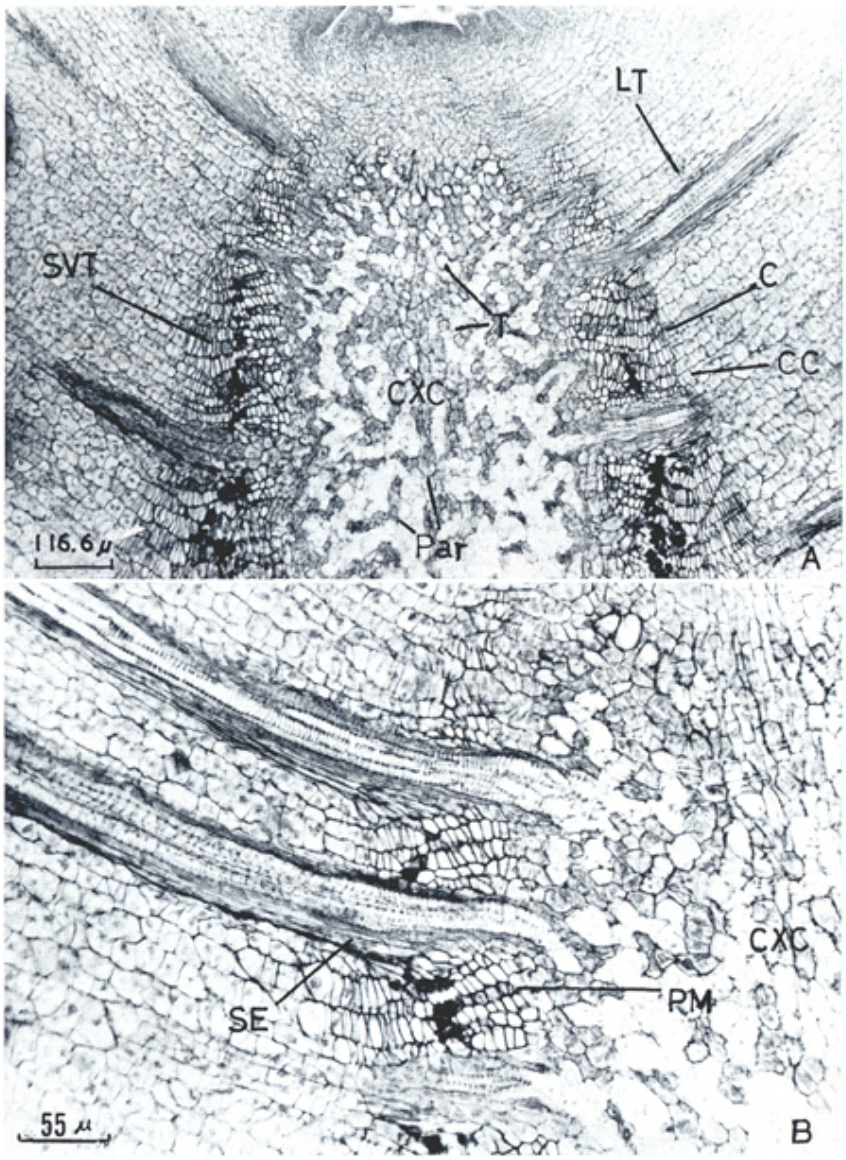

Fig. 1. Median longitudinal sections of corm; A, showing the central vascular tissue near the apical meristem and its adjacent tissuse; B, enlarged view showing the departure of leaf traces from the central vascular tissue. C-cambium; CC-cortical cell; CXC-central xylem cylinder; LT-leaf trace; Par-parenchyma; PM-Phloic mantle; SVT-secondary vascular tissue; T-tracheid.

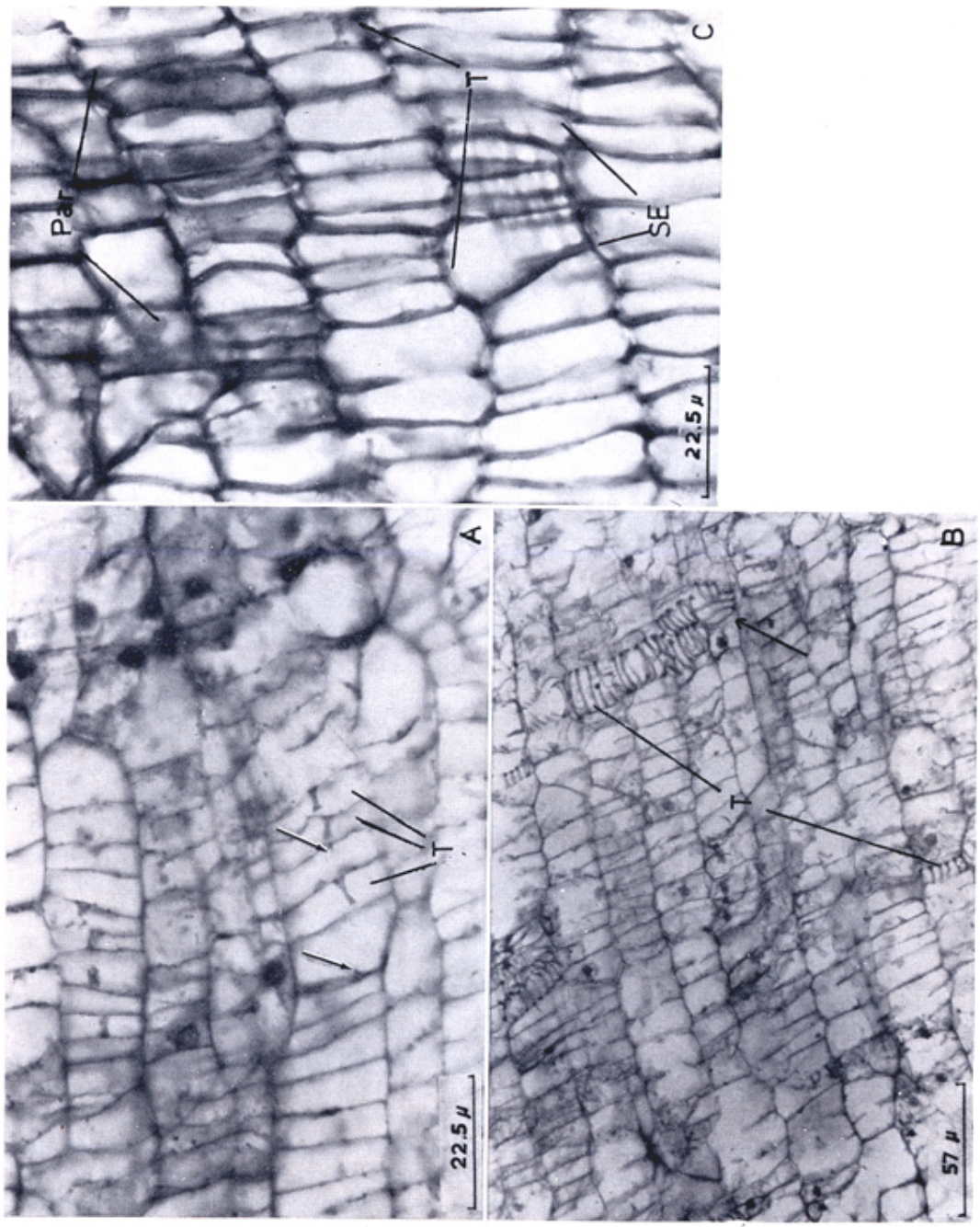

Fig. 2. Photographs from the radial sections through the secondary vascular tissue showing the one-banded tracheids (Fig. A); many-banded tracheids (Fig. B); and parenchyma, tracheids and sieve elements with scalari-pattern of the primary walls.

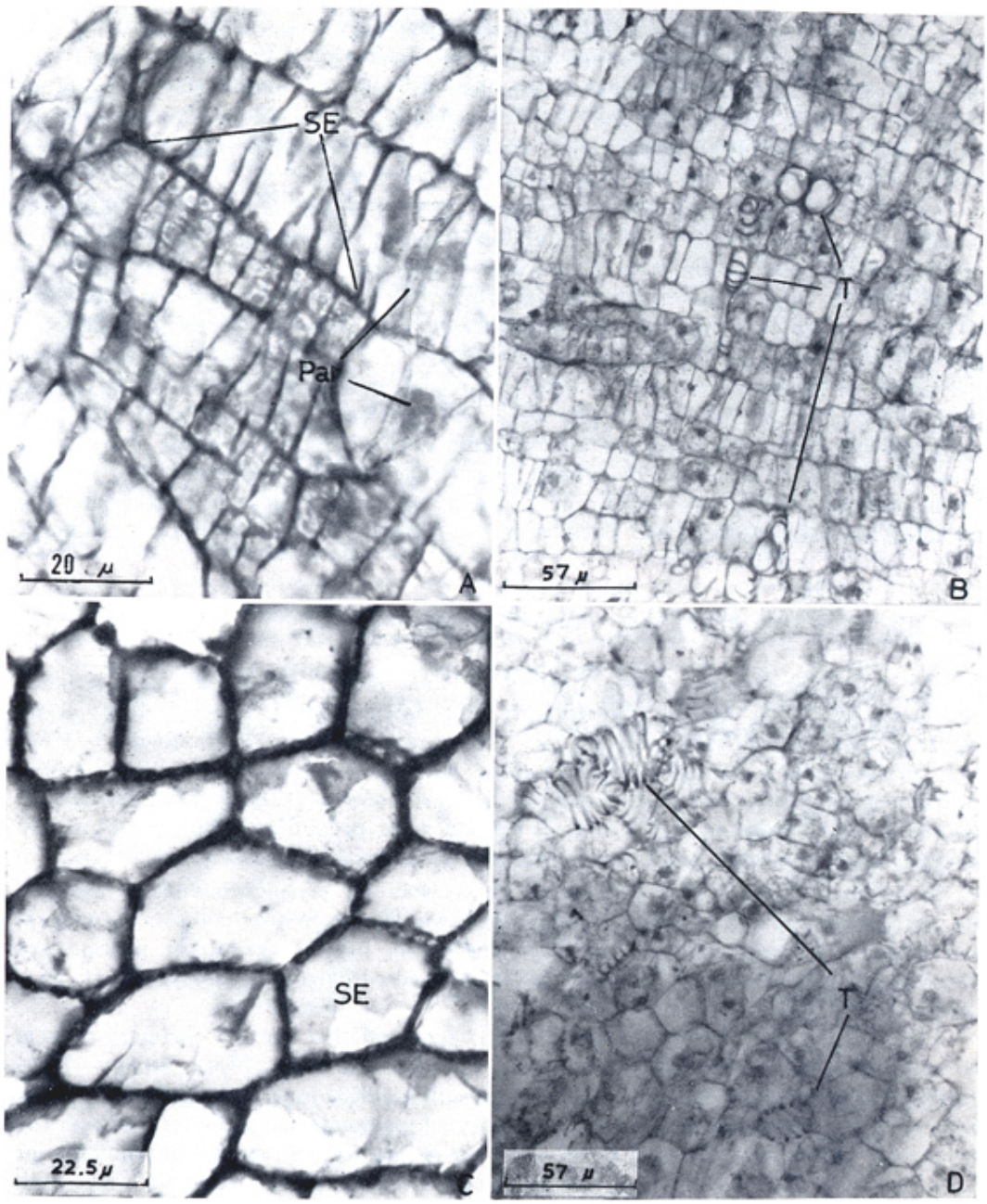

Fig. 3. A, B transverse section of corm. C, D tangential section of corm. SE: sieve elements T: tracheids Par: parenchyma cells

2A, Ac), whereas a few have many-bands which usually appear in the later formed regions of older corms (Figs. 3B, 3D, 4C). The band is golden yellow in the materials stained with tannic acid-iron alum and is red in the safranin-fast green staining materials. The stain effect on the tracheids in the secondary tissue is similar to that in primary vascular tissue, leaf traces and root traces. Lignin is conspicuous. The band in the one-banded tracheids is easily broken during sectioning, so we often observed many empty cells with the remains of a small protrusion (parts of broken bands) in the sections (Fig. 2A, arrows).

Although tracheids are always present, their number is extremely variable depending on the specimens. In one fair sized corm, the tracheids were indistinct, but in another corm of about the same size, tracheids were visible and scattered in the phloem area. Sometimes the secondary tracheids were isolated (Figs. 3B, 3D), but in other cases they formed rather large aggregated patterns (Fig. 2C).

The vascular tissue in the root-and leaf-traces: The sieve elements in the roots and leaves of *Isoetes* are elongated with transverse or somewhat oblique end walls. Sieve areas occur on both the lateral walls and end walls. The tracheids are also elongated but their end walls are always oblique (Fig. 1B). The walls of tracheids have annular or spiral secondary thickenings. The leaf-and root-traces are usually surrounded by one or two layers of parenchyma cells. These parenchyma cells are more or less elongated and parallel to the traces. So it is easy to distingusih them from the cortical cells.

DISCUSSION

Primary vascular tissue: The protostelic stele and lacunated central xylem cylinder have been described by all investigators (Hofmeister, 1862; Farmer, 1890; Scott and Hill, 1900; Smith, 1900; Stokey, 1909; West and Takeda, 1915; Paolillo, 1963; etc). Some of them (Lang, 1915b; Paolillo, 1963) recognized a peripheral and a central portion of the xylem cylinder in several species (*I. lacustris; I. howellii: I. nuttallii*). The tracheids in the central portion were procumbent, but those in the peripheral portion were erect and continuous with the leaf traces (Paolillo, 1963).

In our investigation of *I. taiwanensis* there was no distinct boundary between the peripheral and central xylem. Moreover, we found the tracheids in the central portion were not procumbent but erect, and those in the peripheral portion are slanting, and sometimes procumbent when connected with the tracheids of leaf-traces.

Lang (1915b) and West & Takeda (1915) reported that there is primary phloem surrounding the central xylem cylinder. But Scott & Hill (1900), Stokey (1909) suggested that primary phloem is absent. Paolillo (1963) investigated *I. howellii* and reported that there are one to several parenchyma cells immediately above the peripheral xylem, and that the sieve elements of the phloem are on the outside of these parenchyma cells, but that the primary phloem is obliterated when the secondary growth begins.

Our investigation of *I. taiwanensis* agrees with Paolillo's reports, but the sieve cells and parenchyma cells are not so regularly arranged as those in the species studied by Paolillo. The description of the structure of the cells in the primary vascular tissue has been omitted by other investigators perhaps because it is similar in form and structure to that in other plants.

Secondary vascular tissue: The earliest cambium activity begins outside of the maturated primary sieve elements (Paolillo, 1963).

The inner derivatives of the cambium have been a subject of debate among many investigators. Von Mohl (1845) reported that they are a group of undifferentiated parenchyma cells. Smith (1900) and Stokey (1909) suggested that they are secondary xylem, but others suggest that they are a mixture of sieve elements, tracheary elements and parenchyma (Russow, 1872; Scott and Hill, 1900; West and Takeda, 1915; Paolillo, 1963. etc.).

Weber (1922) examined the sieve elements and identified cellulose, pectic substances and protein mixed with the callose in the wall. He suggested that the sieve elements are only a special type of parenchyma cell and denied its phloic nature. He also reported that the callose deposits were not only on the walls of sieve elements, but were also on the walls of the parenchyma cells in the primary xylem core and leaf-and root-traces. These ideas were supported by Eschrich, (1956) and Currier (1957) who suggested that it may be formed because of the distruptive growth of these cells. Paolillo (1963) added that the ligule cells also have callose, and that the sieve elements accumulated a lot of callose even when there was no disruptive growth. Paolillo reported that there are many characters used to distinguish the sieve elements from parenchyma cells, e. g., callose accumulation, clear cytoplasm, linear chromatic nuclei and thicker walls.

In our investigations, we have found there are parenchyma cells, sieve elements and tracheids. Each with its distinct character, though all similar in shape (Fig. 4). We did not use the staining technique to define the callose. But the sieve areas are distinct when stained with tannic acid-iron alum (Figs. 2C, 3A, 3C). We have found the sieve areas are distributed on all walls except tangential wall, while others have reported that there are the sieve areas distributed on all walls.

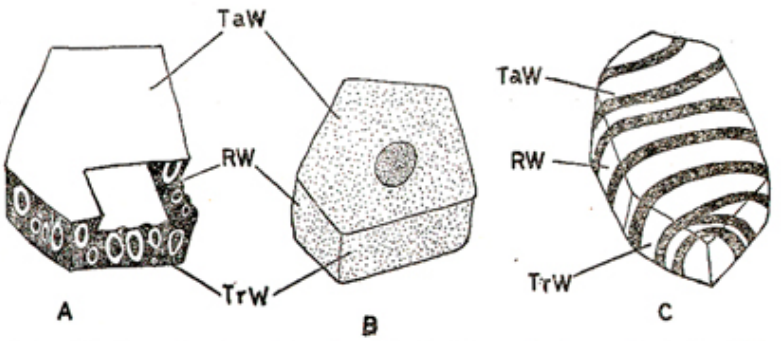

Fig. 4. Drawings showing the three-dimentional structure of sieve elements (A), parenchyma cells (B), tracheids (C) of secondary vascular tissue. TaW: tangential wall, TrW: transverse wall, RW: radial wall.

Paolillo (1963) and others reported that in the young plant of *Isoetes* sp., the cambium only produced sieve elements internally; but in larger plants of *I. howellii*, there were sieve elements, parenchyma cells and tracheids; and in *I. nuttallii*, almost all the inner derivatives of the cambium were sieve elements mixed with a few parenchyma cells.

So they suggested the number of tracheids varies with the specimens, and we agree with this concept. Paolillo (1963) described the tracheid as an empty cell

with ring or helices of secondary wall thickenings. In our investigations, there are two kind of tracheids with different wall thickenings, one has a single band, the other has many bands of secondary wall thickenings. The number of tracheids with one-band is much larger than those with many-bands. The many-banded tracheids are always found in the late formed region of older corms, so this kind of tracheids is usually absent in the young corm.

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