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Early Ontogeny of Vascular Cambium in the Seedling Roots of *Acer saccharinum* L.

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은단풍 (*Acer saccharinum* L.) 유식물의 뿌리에서 유관속 형성층의 초기발생

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ABSTRACT

The origin of cambial initials from procambium was studied in the seedling root of *Acer saccharinum*. In transverse view, the first periclinal divisions of procambial cells occurred just outside of each early metaxylem and resulted in meristematic strips. As root development progressed, the division activities appeared subsequently outside of each late metaxylem and then in pericycle cells opposite the four protoxylem poles. Eventually, such meristematic strips were connected completely each other. Thus, a nearly rectangular shaped meristematic layer in outline was formed outside the xylem in a whole root transectioned. In tangential section, early procambium showed a homogeneous structure consisted of uniform short cells with transverse end walls. However, some of the procambial cells did elongate, whereas others divided transversely. The former became more elongate, tapered, and vacuolated. Finally, they differentiated into fusiform initials. Short cells consisting axial strands divided continuousely in transverse plane and became ray initials, while some short cells elongated and transformed into long cells. The early ontogeny of vascular cambium in *Acer saccharinum* root was interpreted to be established by a gradual process.

INTRODUCTION

The organization of the vascular cambium in root and stem is quite different from that of other meristems, because the vascular cambium has two types of cells, fusiform and ray initials. To clarify the ontogenetic origin of cambial initials, it is necessary to observe the tangential planes as well as transverse ones according to the subequent developmental stages from early procambium to mature cambium.

The ontogeny of stem cambium has been studied by many investigators (Cumbie, 1967; Soh, 1972, 1974a, b; Butterfield, 1976; Soh *et al.*, 1990), while the origin of cambial initials in root has been paid little attention. The authors have only reported that the pattern of origin of cambial initials in *Ginkgo biloba* root differed from that in stem (Soh *et al.*, 1988). However, it has not yet cleared whether the pattern of origin of cambial initals in root also differed from that in stem in many other plants.

Therefore, this paper deals with the origin of cambial initials in the root of *Acer saccharinum*. We have also compared the present results with that in the stem previously reported in the same plant species (Soh *et al.*, 1990).

MATERIALS AND METHODS

The seeds of *Acer saccharinum* L. ripened around the end of May were collected at the campus of Chonbuk National University, Chunju, Korea. The seeds were immediately germinated after droping from tree. On the basis of their shape and weight, the uniform seeds were first selected. The selected seeds were stratified in wet sand at 25± 1℃ for about 7 days. Then germinating seeds with 1 mm-long roots were secondly selected, and four seeds per pot (20×25 cm) were sowed in fine sand. The pots were maintained in a growth room at 65-75% humidity, and 25± 1℃ temperature with 16 hr light each 24 hr period. The seedlings were watered daily.

The harvested seedlings at 1, 3, 7 and 11 days after sowing were measured for their growth state and were used for anatomical studies. The root specimens were fixed in FAA, dehydrated in an tertiary butyl alcohol series and embedded in paraffin. The embedded specimens were sectioned either transversely or longitudinally at 10 μm in thickness and the sections were stained with hematoxylin, 1% aquous safranin and fast green (Berlyn and Miksche, 1976). Although the observation was made through all the length of root, the root base around 5 mm from cotyledonary node was mainly done, because it was inconvenient to examine the twisted roots during their growth.

RESULTS

The root elongation was already completed at the root base because the elongation occurs within about 2 mm from the root tip. This was confirmed by marking with Indian ink every mm interval beginning from the tip as in *Ginkgo* (Soh *et al.*, 1988). For convenience, the developmental process was divided into four stages in related to xylem differentiation: (1) protoxylem stage in 1-day-old seedling, (2) early metaxylem stage in 3-day-old seedling, (3) late metaxylem stage in 7-day-old seedling, (4) early vascular cambium stage in 11-day-old seedling. The development of xylem at each stage was coincident with the chronological age of roots in most cases (Table 1), although some seedlings developed more or less earlier or later.

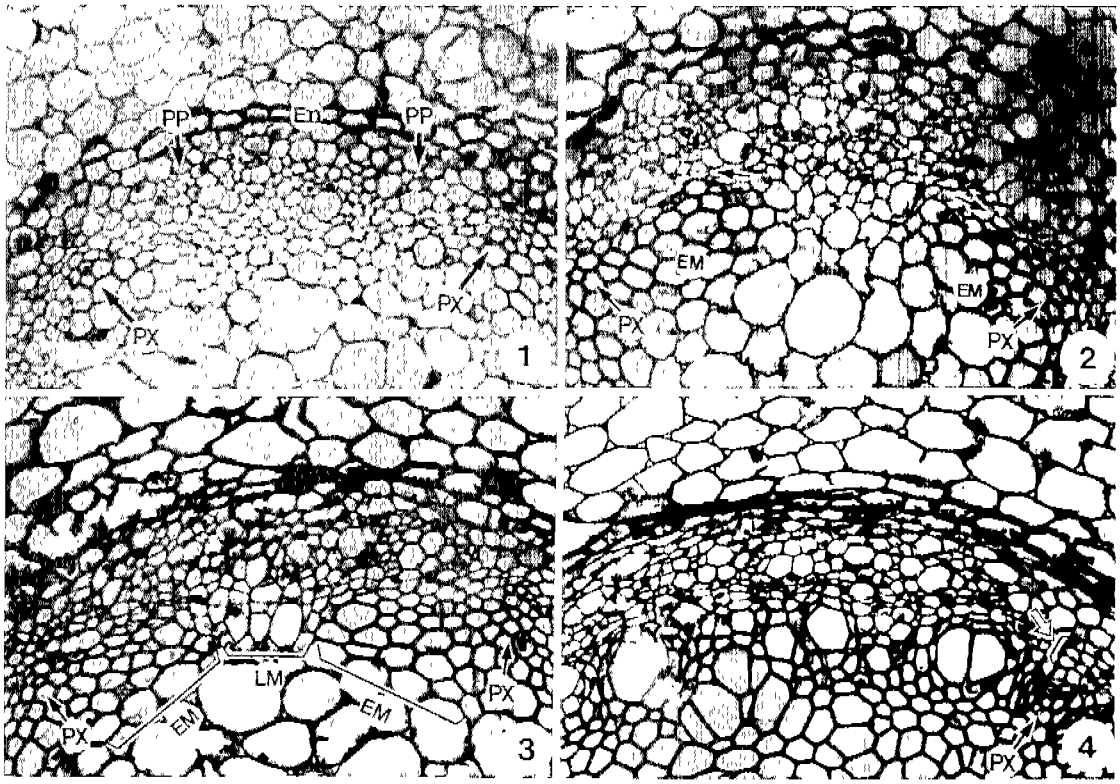
Protoxylem stage. One-day-old seedlings had about 7.5 mm root, and 0.4 mm the first internode in length (Table 1). In transverse view, protophloem differentiated at the periphery of four poles of a nearly rectangular procambium in developing root. And protoxylem differentiated in alternate pattern with phloem bundle at each corner of the procambium (Fig. 1). Thus, a vascular cylinder containing tetrarch xylem was established in three dimensional view. The procambial cells located at the middle part were large and contained starch grains, while the procambial cells between protophloem and future metaxylem were small and divided randomly (Fig. 1). In tangential section, the procambium showed a homogeneous structure composed of uniform short cells with transverse end walls (Fig. 5). Those cells about 69 μm in length (Table 2) divided transversely and longitudinally.

Table 1. Measurement of root with hypocotyl (3 mm) and shoot during growth of *Acer saccharinum* seedlings measured with 30 individuals, respectively.

Measurement		Root	Shoot	
Stage (days)		(mm)	1st internode (mm)	Leaf
Protoxylem	(1)	7.5	0.4	Primodia
Early metaxylem	(3)	17	1.3	Cotyledons unfolded
Late metaxylem	(7)	38	3.1	1st leaf matured
Early vascular cambium	(11)	53	4.2	2nd leaf matured

Early metaxylem stage. In the root base of three-day-old seedling (Table 1), metaphloem outside the metaxylem began to differentiate in transverse view. Because the early centripetal differentiation of the metaxylem was limited near protoxylem pole, there was discontinuity of metaxylem along each margin (Fig. 2). Procambial cells between metaphloem and metaxylem began to divide in periclinal plane and became two isolatded strips of meristematic cells along each margin of procambium or xylem core. Therefore, there are eight meristematic strips outside the procambium or xylem core in a whole root transectioned (Fig. 9). In tangential section, some short cells of the homogeneous procambium in the previous stage elongated, while others did repeated transverse division and had transverse end walls and formed axial strands. Thus, the homogeneous procambium in the protoxylem stage changed into a heterogeneous one with two types of cells (Fig. 6). Long cells were 82 μm in length (Table 2). Short cells formed axial strands which were uniseriate with one to nine cells in height.

Late metaxylem stage. In transverse section of the root base of seven-day-old seedling (Table 1), late metaxylem differentiated centripetally or laterally and each ea-



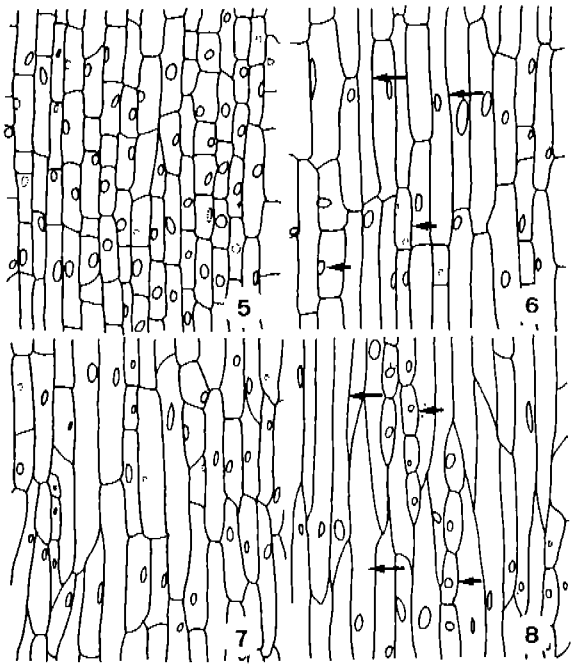
Figs. 1~4. Transverse sections of the root base of developing seedlings (×150). The microphotograph shows about half of tetrach xylem in the vascular cylinder. Fig. 1. Protophloem (PP) and protoxylem (PX) differentiate within endodermis (En) in 1-day-old seedling. Fig. 2. Early metaxylem (EM) differentiates centripetally in 3-day-old seedling. Procambial cells divide periclinally (arrows) outside of the EM, and thus meristematic strips are formed. Fig. 3. Late metaxylem (LM) differentiates between the early metaxylem (EM) in 7-day-old seedling. A meristematic strip formed by periclinal division (white arrow) of procambial cells also appears outside of the LM, and thus the strips formed independently are connected each other. Fig. 4. Meristematic cells formed by pericyclic cells outside of the protoxylem pole are connected with ones formed outside to the EM and LM. This section shows a part of a nearly rectangular meristematic layer which appears in a whole root.

Table 2. Cell length, and height and width of axial strands of short cells in vascular meristems during growth of *Acer saccharinum* seedlings

Measurement		Cell length (μm)		Axial strand (cells)	
Stage (days)		Long cell	Short cell	Hight	Width
Protoxylem	(1)	69± 7.3			
Early metaxylem	(3)	82± 9.3	42± 5.7	1- 9	1
Late metaxylem	(7)	167± 15.9	39± 4.5	2-15	1
Early vascular cambium	(11)	252± 27.6	40± 4.3	7-22	1

rly metaxylem was connected by the late metaxylem. Eventually, nearly cylindrical structure of xylem was formed in three dimensional view (Fig. 3). Meristematic strips were formed by periclinal division of procambial cells outside each late metaxylem. These strips were connected with ones, which appeared previously outside of each early metaxylem. Each meristematic strip between metaxylem and metaphloem consisted of two to four cells in each radial row. In tangential view, the procambium had a clear heterogeneous structure with long cells and axial strands of short cells (Fig. 7). Long cells, about 167 μm in length (Table 2), with tapering ends actively elongated. Some short cells in axial strands elongated and became long cells, while others divided actively in transverse plane. Thus, the height of the axial strands is increased and composed of two to fifteen cells.

Early vascular cambium stage. In transverse section



Figs. 5~8. Tangential sections of the root base of 1, 3, 7 and 11-day-old seedliings in the same stage as in Figs. 1~4 (×150). Fig. 5. Procambium in protoxylem stage has a homogeneous structure composed of short cells with transverse end walls. Fig. 6. Procambium in early metaxylem stage has a heterogeneous structure: one composed of short cells (short arrow) and the others of long cells (long arrow). Fig. 7. Procambium showing more distinct heterogeneous structure in late metaxylem stage. Fig. 8. Vascular meristem has a characteristic of early vascular cambium: two types of initials, fusiform (long arrow) and ray initials in axial strands (short arrow).

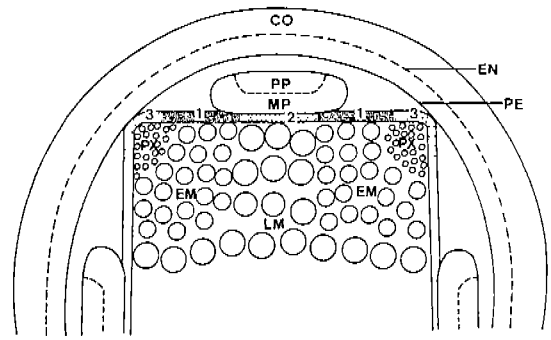


Fig. 9. Diagramatic display of transverse section of a root base showing various stages of xylem differentiation and meristematic strip formation (This diagram shows only about half area of a root containing tetrach xylem). The first periclinal divisions (1) of procambial cells appears outside of the early metaxylem (EM). Thus, there are two strips of meristem along outside of rectagular xylem and then the strips are united into a layer (2) outside late metaxylem (LM). These strips are connected with each other by periclinal divisions (3) of the pericyclic cells (PE) located outside the protoxylem poles (PX). Therefore, the meristematic strips are connected completely, and a continuous layer of meristematic cells are formed just outside the xylem core in transverse section. Co: cortex, EN: endodermis, PP: protophloem, MP: metaphloem.

of the root base of eleven-day-old seedling (Table 1), the xylem inside phloem differentiated more and became nearly cylindrical in three dimensional view (Fig. 4). Pericyclic cells outside the protoxylem poles divided periclinally. Therefore, the meristematic strips were connected completely, and thus a continuous layer of meristematic cells were formed just outside the xylem core in transverse section (Fig. 4). The meristematic strips between metaxylem and metaphloem consisted of three to five cells. In tangential view, long cells about 252 μm in length (Table 2) were stongly vacuolated and have tapering ends due to intrusive growth (Fig. 8). Axial strands of short cells are uniserate and their heights are seven to twenty two cells. The short cells had tapered or transverse end walls. The above structural characteristics reflected that the vascular meristem seemed to be the early stage of vascular cambium.

DISCUSSION

Homogeneous procambium of *Acer saccharinum* stem, which consisted of short cells in early stage in tangential view was consequently transformed into a heterogeneous one consisting of two types of cells: one was long and the other was axial strands of short cells. The former elongated more and became fusiform initials. The latter forming the axial strands did repeated transverse divisions, from which ray initials originated (Soh *et al.*, 1990). This pattern of origin of cambial initials by gradual process is the same as that of the root of the same plants, *Acer saccharinum* in the present study.

Comparison of the ontogeny of the vascular cambium between in stem with that in root in the same plant species has been studied by few workers (Mauseth, 1988; Soh *et al.*, 1988; Soh, 1990; Woods, 1991). We have repor-

ted the early ontogeny of the vascular cambium of stem (Soh, 1972) and root (Soh *et al.*, 1988) in *Ginkgo biloba*. After the elongation of all homogeneous procambial cells of *Ginkgo* root, short cells derived from some long cells through sporadic transverse divisions, while another long cells continously elongated. Such two systems of cells formed in the late procambium of root differed from that in stem, in which those cells were derived from homogeneous procambium in early stage (Soh, 1972). Furthermore, the present results differed from that in *Ginkgo biloba* root (Soh *et al.*, 1988).

In transverse section of *Acer saccharinum* roots (Fig. 9), the first appearance of meristematic strips formed by the periclinal divisions of procambial cells outside the early metaxylem was similar to that in *Ginkgo biloba* (Soh *et al.*, 1988). However, our result differs from that of *Daucus carota* and *Pyrus communis* in which the first appearance of the meristematic strips appeared outside the late metaxylem (Esau, 1940, 1943). Esau(1977) stated that the first periclinal divisions of procambial cells betwen metaphloem and metazylem indicated the initiation of the vascular cambium. However, we could not find two types of cambial initials in the meristematic strips formed by the first periclinal division in tangential view. At the final developmental stage those initials were observed within the vascular cambium through their gradual differentiation.

Many anatomists described the region of the first periclinal divisions in seedling roots and hypocotyls as the early cambium. Such a statement seems to be unclear and result from that they did observe only the transverse sections (Winter, 1932; Bond, 1942; Weaver, 1960; Esau, 1977). The present anatomical study suggested that the examination of the tangential sections with transverse ones would be helpful to better understand on the oigin of the cambial initials from procambium.

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적 요

은단풍 유식물의 뿌리에 있어서 초기의 전형성층으로부터 성숙된 유관속 형성층의 발생과정을 관찰하여, 형성층 원시세포의 기원을 밝혔다. 횡단면 관찰에서, 전형성층 세포의 최초 병층분열은 초기 후생목부의 바깥쪽 가장자리에서, 이어서 후기 후생 목부의 바깥쪽에서 일어나며, 여기서 생긴 분열조직층 들이 서로 연결된다. 다음에는 초생목부의 바깥에 있는 내초세포들이 분열하여, 결국은 절단된 뿌리에서 거의 사각형 모양을 한 뿌리의 중앙에 위치한 목부의 가장자리에 따라서 한층의 분열조직 세포층이 형성된다. 접선면 관찰면에서 초기전형층은 횡단벽을 갖는 짧은 크기의 세포로된 균일 구조를 갖는다. 선형성층의 일부 세포는 신장생장을 하지만 나머지 일부는 횡단분열을 한다. 긴세포는 계속해서 신장생장을 하며 침상말단을 이루며 액포화되면서 방추형 원시세포로 된다. 종축을 이룬 짧은 세포는 계속해서 횡단분열을 하고 일부가 신장생장을 하여 긴세포로 전환된 후에 방상조직 원시세포로 기원된다. 뿌리의 유관속형성층의 발생은 점진적인 과정을 거쳐 이루어지는 것으로 사료된다.

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