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Origin of Callus and Vascular Cambium in Debarked Stem of *Robinia pseudoacacia*

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The calluses formed on the surface of a quarter-girdled *Robinia pseudoacacia* stems have been shown to originate from immature xylem cells and preexisting cambial cells. The callus is not only formed by periclinal and anticlinal divisions of radial cells, but also axial cells. In tangential view, the callus at initial stage showed heterogeneous structure composed of long and short cells and then homogeneous one with short cells. Some cells of homogeneous structure in middle region of callus at early stage is later elongated and others mainly divided in transverse plane. In the result the homogeneous structure becomes into a heterogeneous one. Subsequently, the long cells in heterogeneous structures elongated further and became fusiform initials, and the short cells divided transversely became ray initials. The appearence of homogeneous and heterogeneous structure in the callus on debarked stem without organ elongation is almost similar to that of the structure in the procambium of young stem which is elongating extensively. Eventually, the ontogeny of vascular cambium in wound callus resembles that of a young stem grown normally, although the debarked stem does not grow in length but in girth and the young stem elongates actively. These findings mean that the active intrusive growth of short procambial cells occurs during the differentiation of fusiform cambial cells.

Keywords: vascular cambium, callus formation, debarked stem, *Robinia pseudoacacia* L.

Vascular differentiation may be broadly classified into three types: (1) primary vascular differentiation from procambium in young shoots and roots, (2) secondary vascular differentiation from the vascular cambium in the stem and roots, and (3) regenerative vascular differentiation from vascular meristem by a redifferentiation of callus in the wound stem (Sachs, 1981). It has been reported in transverse view that vascular cambium is differentiated from callus on the debarked stem. However, the origin of callus and vascular cambium in the debarked stem have not yet been reported, especially in tangential observation. Therefore, we have little information on whether the differentiation processes of vascular cambium from callus is the same as from procambium in developing stem or not.

In the course of callus formation and xylem differentiation on exposed surfaces in herby and woody plants, calluses were derived from vascular ray (Dobbins and Fisher, 1986) and the immature xylem element (Sharples and Gunnery, 1933; Steeves and Sussex, 1991), but the amount contributed by the element was negligible in comparison to that produced by ray cells (Brown and Sax, 1962).

In the origin of callus from the exposed surface of the cambial zone of *Trema orientalis* and *Fulbernardia globiflora,* calluses developed from any of the undifferentiated centripetal products of the vascular cambium, but the kind of tissue contributing to callus initiation depended upon the species and on the histology of the cambial zone (Noel, 1968, 1970). In *Mikania,* there was massive callus formation from the large multiseriate rays in wound stem. In *Eucommia,* formation of callus derived from immature xylem which was swelled, proliferated and spread laterally under the surface layer. It gradually joined together with the neighboring ray cells and also mixed with some other cells derived from the immature xylem (Li and Cui, 1988).

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In tangential view, the procambium at early stage shows a homogeneous structures (Esau, 1943, 1965; Soh, 1972, 1974a, b) and later stage has two distinct systems, one made of long cells by elongation, the other of short cells by repeated transverse divisions during active internodal elongation (Soh, 1974a, b), and then differentiated into vascular cambium composed of fusiform initials with tapered end walls, and almost isodiametric ray initials. The examination controlling the formation and activity of the procambium and cambium showed that there was no special organizing stimulus responsible for the differentiation of the cambium in a study on regeneration after wounding (Fahn *et al.*, 1972). Therefore, it has been suggested that procambium and cambium are the same meristems at different stage (Fahn *et al.*, 1972; Soh, 1972, 1974a, b; Butterfield, 1976; Larson, 1976). Thus the vascular meristem is conveniently divided into procambium and cambium.

In this context, whether the ontogeny of vascular cambium from the callus formed on debarked stem is the same as that from procambium of young stem remains to be examined. There are great differences between mature and developing stems in growth pattern. The mature stem does not grow in length but developing stems elongate actively. The origin and development of vascular cambium in those stems with contrast growth pattern should be justified in developmental anatomy. This study was, therefore, conducted to examine the origin and development of callus and vascular cambium in debarked stems in tangential plane.

MATERIAL AND METHOD

Robinia pseudoacacia trees of about 5 y old were growing on a hill in Chungnam National University, Taejon, Korea. Pieces of approximately 50 by 20 mm bark were stripped off the main trunk at chest height with a chisel and grafting knife. A quarter-debarked trunks were wrapped with a semi-transparent plastic sheet immediately after debarking. Sample blocks (1×1 cm) were then removed at various intervals with a chisel. The blocks were fixed in FAA, and embedded in paraplast at the embedding center according to conventional procedures. Each block was sectioned with a rotary microtome at 10 μm in thickness. Serial sections were stained with hematoxylin, safranin and light green (Sass, 1971) then observed by a light microscope (Leitz Wetzlar).

RESULTS

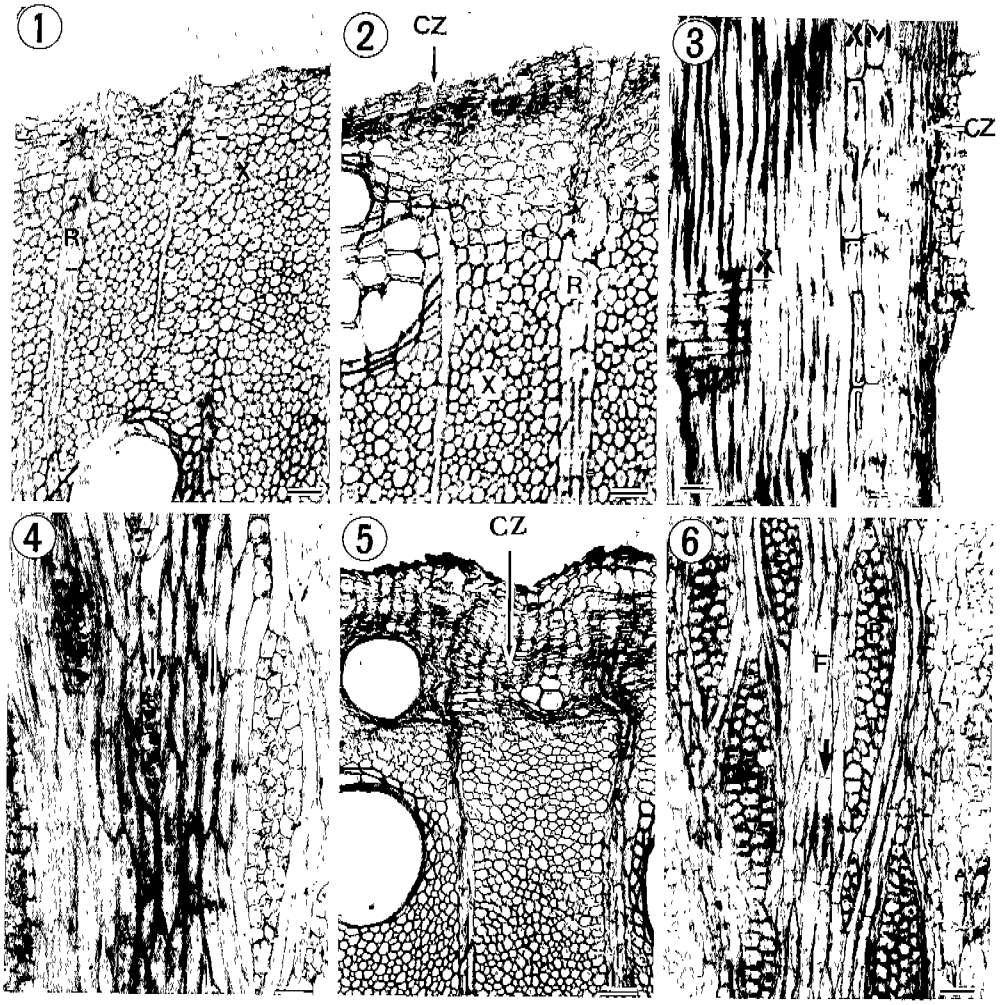
Callus formation

The girdled tree, *Robinia pseudoacacia* could be killed, and did not observed the formation of callus (Fig. 1). However, in the case of a quarter-girdling the tree remained alive for a long time and formed callus on wound surface. In transverse view, we observed some differentiating elments and ray cells of xylem inside a vascular cambium immediately after debarking (Fig. 2). In radial view, a vascular cambium consisted of radially flattened fusiform initials, and ray with erect rectangular cells (Fig. 3). While, in tangential view, vascular cambium was organized into two types of cells, one composed of fusiform initials with tapering end walls, averaging 210 μm in length, the other of ray initials, 1-19 cells in height, 1-4 cells in width. They stained deeply, revealing prominent nuclei (Fig. 4).

Five d later debarked stems initiated formation of callus by cell enlargement, and periclinal divisions of both ray cells and axial cells. The callus cells had a slightly irregular arrangement (Fig. 5). Callus derived from ray cells protruded more than the axial parenchyma of immature xylem element. Therefore, it appeared rather irregular in thickness. In tangential view, remnant fusiform initials and axial parenchymal cells repeated transverse divisions and shortened to 70 μm in length with transverse end walls. Also remnant ray initials and ray cells divided periclinally and anticlinally (Fig. 6). Consequently, heterogeneous structures composed of long and short cells gradually become transformed into homogeneous structures with short cells. In tangential or radial view, the long cells of fusiform initials and fusiform elements shortened by divisions mixed with cells derived from ray initials and ray cells of immature xylem (Fig. 7).

Development of vascular cambium

Developmental stages of vascular cambium derived from callus were conveniently classified into early (14 d after debarking), late stage (21 d after debarking) of vascular meristem, and completion stages (34



Figs. 1-4. Transverse, radial and tangential sections of *R. pseudoacacia* immediately after debarking. Fig. 1. Exposed xylem (X) by debarking. Fig. 2. Cambial zone (CZ), xylem (X), xylem mother cells (XM) and ray cells (R). Fig. 3. Xylem (X), xylem mother cells (XM) and cambial zone (CZ). Fig. 4. Showing fusiform (long arrow) and ray initials (short arrow). **Figs. 5, 6.** Transverse and tangential sections of *R. pseudoacacia* 5 d after debarking. Fig. 5. Periclinal divisions of preexisting cambial zone (CZ). Fig. 6 Periclinal divisions (arrow) of fusiform initial derivatives (F), and enalrgement and proliferation of ray initial derivatives (R). Bar=50 μm.

d after debarking) of vascular cambium.

Early stage of vascular meristem: Fourteen d after debarking, callus was about 500 μm in thickness, some cells of callus was partly divided by periclinal divisions in transverse view (Fig. 8). The periclinal division could be initiated vascular meristem. In tangential view, vascular meristem in callus at this stage was a homogeneous structure of short cells with transverse or round end walls. These cells attained an average length 50 μm in axial plane (Fig. 9). In the radial view of callus, the vascular meristem consisted of upright, or erect cells with truncated end walls (Fig. 10).

Late stage of vascular meristem: Twenty-one d after debarking, callus, 625 μm in thickness underwent less proliferation than the former stages. Newly formed vascular meristem had 3 cells of radial rows by periclinal divisions of the preexisting cells in outer xylem and was observed cork cambium (Fig. 12). In tangential view, homogeneous structures of vascular meristem became into heterogeneous one composed of long cells with tapering end walls by active elongation and short cells dividing transversely. The mean length of the long cells was 140 μm, while short ones were 1-7 cells in height and 1-2 cells in width (Fig. 11). In radial view, radially flattened vascular meristem was observed in the outer

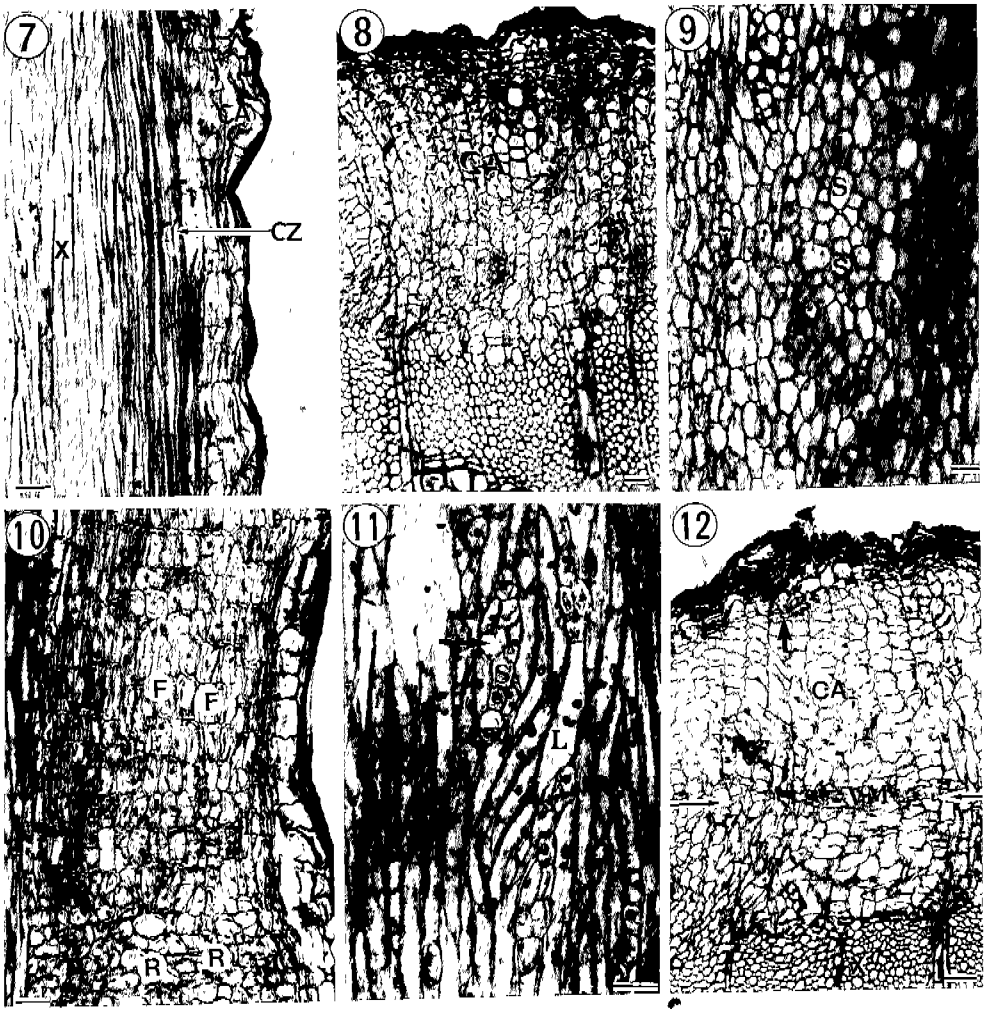


Fig. 7. Radial sections of *R. pseudoacacia* 5 d after debarking. Anticlinal divisions of cambial zone (CZ), xylem (X) and callus (CA). **Figs. 8-10.** Transverse, tangential and radial sections of *R. pseudoacacia* 14 d after debarking. Fig. 8. Showing the callus composed of isodiametric cells. Fig. 9. A homogeneous structure composed of short cells (S) at early vascular meristem stages (arrow). Fig. 10. Extensive callus pad by division of fusiform initial derivatives (F) and ray initial derivatives (R). **Figs. 11, 12.** Tangential and transeverse sections of *R. pseudoacacia* 21 d after debarking. Fig. 11. Elongation of long cells (L). Fig. 12. Newly formed vascular meristem (VM) and newly formed cork cambium (arrow) within the callus (CA). Bar=50 μm.

xylem (Fig. 13).

Completion stage of vascular cambium: Thirty-four d after debarking, the thickness of callus was 1000 μm. Vascular cambium from which produced secondary xylem inward and secondary phloem outward exhibited radial seriation with 5 cells in each row in transverse view (Fig. 14). In tangential view, the mean length of long cells elongated to 162 μm. The cells showed tapering end walls, marked nuclei, deep staining of protoplasm. The height of short cells in axial files was 1-13 cells, and the short cells were 1-4 cells in width (Fig. 15). Thus vascular cambium gradually differentiated from vascular meristem in the former stage, showed the characteristic of differentiating vascular tissue in this stage. In radial view, vascular cambium with radially flattened wall was observed (Fig. 16).

DISCUSSION

It was difficult to observe the origin of callus formation in transverse plane. To elucidate the origin of callus and vascular cambium in detail we demon-

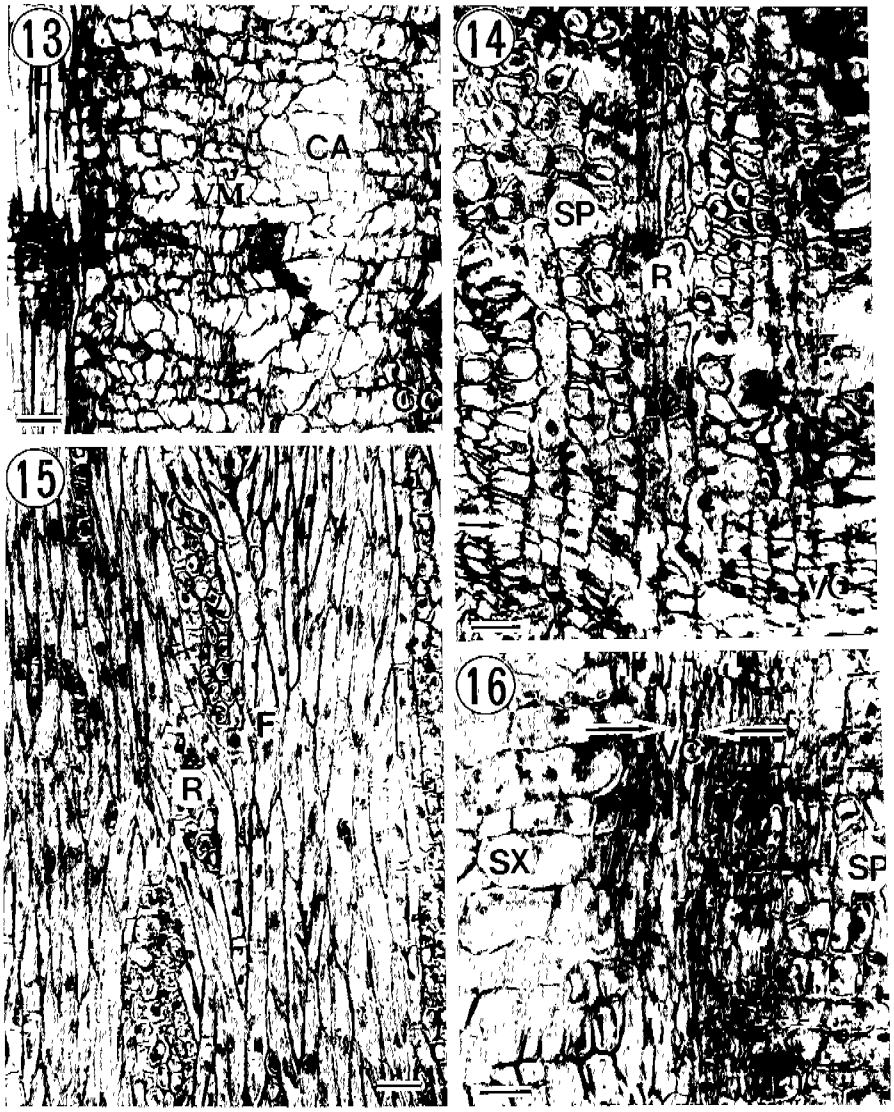


Fig. 13. Radial sections of *R. pseudoacacia* 21 d after debarking. Vascular meristem (VM) and cork cambium (CC). **Figs. 14-16.** Transverse, tangential and radial sections of *R. pseudoacacia* 34 d after debarking. Fig. 14. Vascular cambium (VC), secondary phloem (SP), and ray cells (R). Fig. 15. Vascular cambium composed of fusiform initials (F) with tapering end walls and ray initials (R). Fig. 16. Differentiating secondary xylem (SX), secondary phloem (SP) and vascular cambium (VC). Bar=50 μm.

strated the origin in both tangential and transverse planes. The present study suggested clearly the participation of remnant fusiform initials as well as ray initials and their derivatives in callus formation. Our conclusion is almost similar to those of Noel(1968) in the origin of callus formation of debarked stem in transverse view, but different from the process of the origin. He could not trace the process because of only observation in transverse sections.

In *Eucommia*, the callus originated from ray parenchymal cells of immature xylem, and axial parenchymal cells also participated in callus formation 3 d after wounding (Kang and Soh, 1993b). In *Robinia*, callus was initiated 5 d after wounding. So callus initiation was unique depending upon the species. In *Eucommia*, cell division for callus formation after debarking lasted for 10 d, while in *Robinia*, it lasted for 16 d (Kang and Soh, 1993b). The period of cell division for the callus formation also depends upon the species (Warren Wilson and Warren Wilson, 1984; Noel, 1968).

In both *Robinia* and *Eucommia*, a new vascular

cambium is developed within the homogeneous callus formed by transverse divisions. It was found that early vascular meristem formation took place in 21 d in *Robinia* while in 13 d in *Eucommia* (Kang and Soh, 1993b). The new vascular cambium formation is usually independent upon the amount and the rate of callus production (Soe, 1959).

In transverse view, late developmental stage of procambium consisted of 3 radial rows of cells. The early developmental stages of vascular cambium consisted of 3-4 radial rows of cells in intact stem. But, in late stages of debarked stem, vascular meristem appears in 2-3 radial rows, and 2-8 radial rows of cells in *Eucommia* hypocotyl (Kang and Soh, 1993 a). According to species, there is a little difference in the number of radial rows of cells.

There are contrasting differences between mature stem growing in girth and young stem showing active elongation in growth pattern. The differentiation of vascular cambium from procambium completes at the end of internodal elongation in many species (Soh, 1990). Thus the differentiation of fusiform cambial cells from short procambial cells occurs during internodal elongation but differentiation from callus cells occurs in mature stem without elongation. In the result there are similarities in the differentiation of fusiform cambial cells from short cells of callus and procambium accompanying active intrusive growth of cells. Therefore, it is clear that the intrusive growth of cells in fusiform cambial cell differentiation is indispensable process.

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剝皮된 아까시나무의 줄기에서 캘러스 形成 및 維管束 形成層의 起源

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적 요

박피된 아까시나무 줄기의 표면에 형성되는 캘러스의 기원은 미성숙 목부 요소, 또는 기존의 유관속 형성층 세포 등으로부터 기원된다. 캘러스는 기존의 유관속 형성층의 방사계 세포 뿐만 아니라 주축계 세포들이 병층 및 수층 분열하고 확장되어 분열하면서 발생되며, 접선 단면에서 초기의 캘러스는 긴 세포와 짧은 세포로 구성된 비균일 구조를 보이나 후기에는 짧은 세포로 구성된 균일 구조를 보인다. 이 균일 구조를 구성하는 일부 세포들이 유관속 형성층 형성 후기에 신장되며, 나머지 세포들은 주로 횡단 분열된다. 그 결과 균일 구조는 비균일 구조로 된다. 비균일 구조의 긴 세포는 좀 더 신장되어 방추형 원시세포로, 짧은 세포는 병층분열하여 방사조직 원시세포로 분화된다. 길이 신장이 일어나지 않는 박피된 줄기에서 형성된 캘러스에서의 균일 구조에서 비균일 구조로 변형되는 특징은 절간이 신장중인 유식물 줄기에서의 전형성층 구조와 유사하였다. 이 결과는 짧은 전형성층 세포의 심한 관입생장이 방추형 원시세포의 분화 중에 일어난다는 것을 의미한다.

주요어 : 유관속 형성층, 캘러스 형성, 박피된 줄기, 아까시나무

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