식물학회지 제31권 제 4 호
Korean J. Botany 31(4): 289-298(1988)

Origin of the Vascular Cambium in the Developing Hypocotyl of *Glycine max* Seedling

Kang, Kyung Duck and Woong Young Soh
(Department of Biology, Chonbuk National University, Chonju)

大豆 幼植物의 胚軸에 있어서 維管束形成層의 起源

姜 京 德·蘇 雄 永
(全北大學校 自然科學大學 生物學科)

ABSTRACT

The pattern of elongation in the developing hypocotyl of *Glycine max* shows that the elongation generally proceeds from base to the cotyledonary node in acropetal direction, although earlier elongation takes place through the entire hypocotyl. Because the differentiation of the vascular cambium in the hypocotyl advances also acropetally, it can be seen that the acropetal wave of hypocotyl elongation is associated with the acropetal differentiation of the cambium in the hypocotyl. The elongation of procambial cells occurs not only during active elongation but also after cessation of elongation of the hypocotyl. In tangential view, the procambium of the hypocotyl in early stage has homogeneous structure composed of short cells. Subsequently, these procambial cells elongate actively and then become elongated long cells. These long cells eventually become fusiform initials, while some of elongated long cells are transversely divided and then converted into ray initials. The characteristics of the vascular cambium are entirely acquired some time after hypocotyl elongation is completed, and the transition from procambium to vascular cambium in the hypocotyl is a rather gradual process.

INTRODUCTION

The internodal growth is related to the development of the primary vascular tissues and the vascular meristem. Therefore, it is necessary to examine the developmental pattern of internode in order to understand the development of the primary vascular tissues and vascular meristem. Considerable variations are observed on the developmental pattern of internodes in such plants as *Lycopersicon* (Thompson and Heimsch, 1964), *Helianthus* and *Syringa* (Wetmore and Garrison, 1966), *Helianthus* (Garrison, 1973), *Phaseolus* (Enright and Cumbie, 1973), and *Hoheria* (Butterfield, 1976). Since the same developmental goal is able to be attained in different

developmental sequences, it is supposed that the variations occur in the different region of a developing internode at any time.

The transition from procambium to cambium is associated with the internodal elongation in seed plants. It is commonly known that the characteristics of the vascular cambium occur during the final stage of internodal elongation (Eames and MacDaniels, 1947; Catesson, 1964; Philipson and Ward, 1965; Enright and Cumbie, 1973; Butterfield, 1976). However, little research has been undertaken to describe the primary–secondary transition related to the hypocotyl elongation. This study was conducted in *Glycine max* seedling to investigate the pattern of hypocotyl development and the relation of the hypocotyl elongation to the ontogeny of the vascular cambium.

MATERIALS AND METHODS

Glycine max (L.) Merrill seeds (about 0.4 g in weight) were soaked for 24 h in tap water and maintained in a dark room at 20℃. After the seed coats were ruptured, seeds with root of 3 mm in length were selected and planted in pots containing fine sands (1–1.5 mm in diameter). The pots were maintained in a growth chamber at 23–25℃, in relative humidity of 60–70%, and for 16 h of light a day (7,000 Lux measured at the shoot tip).

The straightening hypocotyls of the 4–day–old seedlings reach to a length of 55 mm. Each one of 100 hypocotyls selected for measurement was graduated into five equal segments marked by India ink at 11 mm intervals from the base. The marking and measurement of segments were started from the stage of later primary growth, four day after planting (Table 1).

The seedlings were harvested for anatomical study on the first day after planting and at successive four day intervals. The seedlings harvested provided a complete range of material to relate hypocotyl development to the vascular differentiation in hypocotyl segment V (Fig. 1). The sampled specimens were fixed in FAA, dehydrated by butylalcohol series, embedded in paraffin, and mounted on blocks. The mounted specimens were cut transversely or longitudinally with a rotary microtome at 10 μm. The sections were stained with hematoxylin, safranin, and fast green (Sass, 1971). On the other hand, observation was also made through the serial transverse or tangential sections in order to confirm whether the developmental process of hypocotyl tissues sampled along the order of chronological age is coincident with the process from the hypocotyl base to cotyledonary node of a seedling or not.

RESULTS

The young seedlings emerge on the ground about four days after planting. Soon after the cotyledons of seedlings are unfolded the first pair of leaves become visible at the tip of the shoot axis. The first two leaves are opposite and simple, but successive leaves produced

thereafter are alternate and compound with three leaflets.

Pattern of hypocotyl development. In the early stage of growth in observation of the present study, the length of hypocotyl segments is almost similar to that of the final in the segments Ⅰ and Ⅱ, and increases only slightly in the segment Ⅲ (Fig. 1). The hypocotyl appparently elongates in the segment Ⅳ and exhibits the most prominent elongation in the segment Ⅴ around five days after planting. Therefore region of the hypocotyl just below the cotyledons elongates actively while successive lower regions exhibits gradually slight elongation (Fig. 1). As a consequence, the development of successive segments occurs in such a manner that a wave of growth progresses from the base in an acropetal direction. The growth occurs throughout the entire hypocotyl in earlier stage than seedling emergence on the ground but immediately ceases in basal region, and continues in uppermost region for some time. The hypocotyl elongation has completed around seven days after planting.

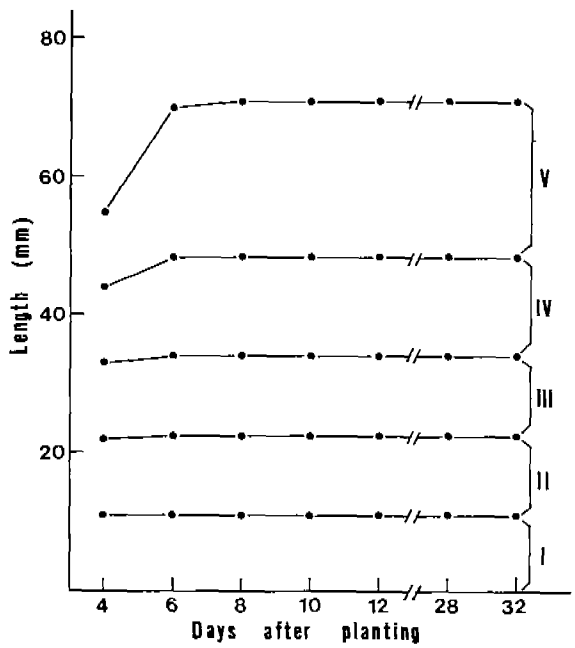

Fig. 1. Pattern of growth of hypocotyl segments marked with India ink at 11 mm intervals. The young hypocotyl, 55 mm in length, was graduated into five equal segments from base (I) to uppermost region(V) of the hypocotyl.

Differentiation of the vascular tissue and meristem. Among the intercotyledonary bundles in the segment V, the vascular meristem in the larger central bundle was selected consistently for observation, and observed tangentially or transversely. Developmental processes of the hypocotyl in the present study were divided conveniently into four stages. The earlier two stages are the early and late primary growth, the sequent stage is the completion of primary growth, and the last stage is the initiation of secondary growth (Table 1).

Table 1. Mean cell length of vascular meristems during growth of *Glycine max* hypocotyl measured with 30 cells in each stage

Measurement / Stage (days)	Segment length (mm)	Cell length (μm)	
		Long cell	Short cell
Early primary growth (1)		50.88± 6.39	
Later primary growth (4)	11	66.36±14.17	
Primary growth completion (8)	27	148.68±42.25	
Secondary growth initiation (12)	27	205.08±40.23	91.79±19.82
Advanced secondary growth (32)	27	242.37±44.29	90.85±23.01

The early primary growth

The hypocotyl of 15 mm in length is elongating in the stage of one day after planting (Figs. 2 and 3). At this stage the vascular system consists of discrete bundles: they are interrupted by narrow interfascicular regions. At the outside of the vascular bundles, the primary phloem differentiated as groups of small cells is separated from the parenchymatous cells of cortex. The primary xylem differentiates at the inside of procambium. There are only a few tracheary elements in the protoxylem (Fig. 3). In transverse section the procambial cells in the vascular bundle show radial seriations consisting of one or two cells in each row. In tangential view, the procambium has homogeneous structure composed of short cells with transverse end walls. The cells are divided transversely as well as tangentially and are average 51 μm in length (Fig. 8; Table 1). Their nucleus and cytoplasm are conspicuously stained.

The later primary growth

The hypocotyl of four-day-old seedling is 55 mm long, elongated actively and straightens up. In transverse view, the vascular bundle is interrupted by narrow interfascicular regions as in the previous stage (Fig. 4). The primary phloem is separated from the cortex by enlarged parenchymatous cells with darkly stained substances. Some vessel elements of protoxylem are crushing, and differentiating metaxylem has two to five cells in radial rows. The phloem fiber begins to be differnetiated at the outer region of the phloem. The radial rows of procambial cells characterized by repeated periclinal divisions are clearly discernible as well as those of vessels in primary xylem (Fig. 4). The procambium consists of two or three cells in each radial row. The radial cell divisions in the vascular meristem are observed in this stage (Fig. 4,

arrow). In tangential view, the procambium is essentially still homogeneous, although some of procambial cells begin to elongate. They have mainly transverse end walls (Fig. 9), and are average 66 μm in length.

The completion of primary growth

The eight-day-old seedling has average 76 mm in length of the hypocotyl and its elongation is ceased. In transverse view, metaxylem elements are clearly differentiated more than in the former stage (Fig. 5). Periclinal divisions of meristematic cells occur more actively than in the former stage and begin to spread tangentially. As a result, the vascular meristem consists of radially flattened two to four cells in each radial row. In transverse view, therefore, this repeated periclinal divisions of the vascular meristematic cells are almost similar to the activity of the vascular cambium. In tangential section, the vascular meristem shows almost homogeneous structure. However, in contrast with the previous stage, the cells of the vascular meristem have tapering ends through their elongation and intrusion between neighboring cells (Fig. 10). And some cells of the meristem begin to divide transversely producing short cells at the end of cells (Fig. 10, arrow). The average length of elongated cells is 148 μm.

The initiation of secondary growth

The hypocotyl length of the 12-day-old seedling is the same as that in the former stage. In the interfascicular region near the vascular bundle some cells begin to divide periclinally. The radially flattened cells of the vascular meristem show almost the same appearance as those in the former stage, and exhibit radial rows with two to four cells. In tangential view (Fig. 11), the long cells of the vascular meristem have clearly tapering end. Some of the elongated long cells begin to divide transversely showing transverse end walls. Thus, the vascular meristem is organized into two systems composed of long cells and short cells in the axial strand. These cells are average 205 μm and 92 μm in length. The strand of short cells is one cell in width and one to three cells in height. The appearance of these two types of cells seems to indicate the initiation of the vascular cambium.

Further advanced structure of the vascular cambium in 32 day-old seedling is shown in Figs. 7 and 12. In transverse section, the general appearance of the vascular cambium almost resembles that of 12-day-old seedling. In tangential section, the vascular cambium shows heterogeneous structure organized into two distinct systems (Fig. 12). The ray initials in axial strands have transverse end walls and average 91 μm in length. The fusiform initials have clearly tapering end and are average 242 μm in length, and begin to show storied arrangement by repeated radial longitudinal division. As seen in transverse section, the region of the hypocotyl between its base and 25 mm level have the exarch xylem as in root. The vascular arrangement of the hypocotyl between level of 25 mm and 38 mm gradually changes into that of the shoot. Thus, the upper part of 38 mm level of hypocotyl has the endarch xylem. The differentiation of the vascular cambium in the hypocotyl progresses from base to cotyledonary node in acropetal direction. It was found from serial transverse or tangential observation that the vascular cambium already differentiated in the middle part of segment Ⅳ but not in the

middle part of segment V in 8-day old seedling hypocotyl (Fig. 13). Consequently, the development of the vascular cambium in the hypocotyl sampled through chronological age on each stage described in the above is almost the same as the development in young seedling hypocoyl sampled from the base to the cotyledonary node.

DISCUSSION

The growth pattern of the hypocotyl in seedlings of *Glycine max* reveals that the elongation of the hypocotyl takes place more actively in the region just below cotyledonary node than in the base. This developmental pattern is very similar to that of internodes in such plants as *Helianthus* and *Syringa* (Wetmore and Garrison, 1966), *Helianthus* (Garrison, 1973), *Phaseolus* (Enright and Cumbie, 1973), and *Hoheria* (Butterfield, 1976). The elongation of the successive segments of *Glycine max* hypocotyl progresses upward from the base to the cotyledonary node of hypocotyl. A similar acropetal wave of elongation has been also observed in the growth of the hypocotyl in seedlings of *Brassica* (Havis, 1940) and bean (Klein and Weisel, 1964), and the internode of *Helianthus* (Garrison, 1973) and *Phaseolus* (Enright and Cumbie, 1973).

The differentiation of the vascular cambium in *Glycine max* hypocotyl also advances acropetally. Therefore, in the hypocotyl of *Glycine max*, acropetal progression of elongation is associated with that of cambial ontogeny. In *Populus*, the cambial-procambial sequence proceeds also acropetally and continuously (Larson, 1976). During the elongation of the hypocotyl in *Glycine max*, the active elongation of procambial cells occurs simultaneously and the elongation of cells also proceeds subsequently after cessation of hypocotyl elongation (Table 1). Thus, the elongation of cells after cessation of hypocotyl elongation would be due to intrusive growth of procambial cells. The elongation of procambial cells after cessation of root elongation also occurred in *Ginkgo* (Soh *et al.*, 1988). Differently from the result of *Glycine* (Table 1), it is shown that the length is shorter in cambial fusiform initials than in long cells of the procambium at the end of primary growth in *Phaseolus* (Enright and Cumbie, 1973).

The characteristics of the vascular cambium in *Glycine max* are acquired some time after the completion of hypocotyl elongation. In this respect, the cambial initiation of *Glycine* is similar to the case of *Acer*: the appearance of its cambium has acquired some time after internodal elongation has ceased (Catesson, 1964). In many plants, it is known that the characteristics of the vascular cambium occur during the final stage of internodal elongation (*Lycopersicon*, Thompson and Heimsch, 1964; *Canavalia*, Cumbie, 1967; *Phaseolus*, Enright and Cumbie, 1973; *Hoheria*, Butterfield, 1976). In *Sequoia*, however, the vascular cambium arises in the internodes that are still elongating vigorously (Sterling, 1946).

The radial seriation of cells by repeated periclinal divisions is observed in the procambium as well as in the cambium of *Glycine max* hypocotyl, and the cell number in a radial row of the vascular meristem remains almost constant. This phenomenon on the cell number is similar to the case of many other plants (Fahn *et al.*, 1972; Enright and Cumbie, 1973; Soh, 1974b;

Butterfield, 1976), although there is a few dissimilar cases on *Aucuba* and *Nicotiana* (Esau, 1965).

In tangential section, the procambium in *Glycine max* hypocotyl shows homogeneous structure composed of short cells with transverse end walls in the early stage of hypocotyl development. This is similar to that of the internode of some other plants including *Nicotiana, Robinia* and *Phaseolus* (Esau, 1938; Soh, 1972, 1974a, 1974b; Enright and Cumbie, 1973). In *Glycine,* some of the procambial cells with transverse end walls begin to elongate in the later stage and then all of the procambial cells become elongated long cells at the end of primary growth. Thus, during primary growth, all of the procambial cells are elongated actively so that the procambium consists of only one system with long cells. In this respect, the procambium structure of *Glycine* is similar to the cases of the internode of *Acer* (Catesson, 1964) and *Aucuba* (Soh, 1974a).

Eventually, some of the elongated long cells divide transversely and have transverse end walls (Fig. 11). In consequence, the vascular meristem begins to show two distinct systems with long and relatively short cells, and attains the characteristics of the vascular cambium. The first cambium in *Glycine* hypocotyl shows the arrangement of ray initials with one cell in width and one to three cells in height. However, the ray initials of the first cambium are axially arranged in their considerable height in some leguminous plants, *Canavalia, Phaseolus,* and *Robinia* (Cumbie, 1967; Enright and Cumbie, 1973; Soh, 1974b), and at the end of the primary growth in *Phaseolus* the axial strands of short cells are biseriate (Enright and Cumbie, 1973). The first cambium in *Glycine* shows clearly nonstoried arrangement of fusiform initials, while somewhat advanced cambium in 32 day-old seedling has storied arrangement of fusiform initials. In *Phaseolus* and *Aeschynomene,* even at the end of their primary growth the long cells of the procambium arrange in storied manner (Enright and Cumbie, 1973; Cumbie, 1984).

The authors wish to express their sincere thanks to Professor N. Hara of the University of Tokyo for his critical reading of the manuscript.

摘 要

生長 中인 大豆 幼植物에 있어서 胚軸의 伸長은 初期에는 胚軸 전체에 걸쳐서 일어 나다가 基部에서 부터 子葉節로 向해 求頂的 伸長이 진행된다. 胚軸에 있어서 維管束形成層의 分化도 역시 求頂的으로 진행되므로 胚軸의 伸長과 形成層의 分化는 關聯이 있는 것으로 보인다. 胚軸의 伸長이 활발하게 진행되는 동안 그리고 伸長이 멈춘 後에도 前形成層細胞는 伸長을 하게 된다. 切線斷面의 경우, 初期段階의 胚軸에 있어서의 前形成層은 짧은 細胞들로 구성되어 同質的인 構造를 이루고 있다. 계속해서, 前形成層細胞들의 伸長이 활발하게 진행되어 긴 細胞들로 된다. 결국 이 긴 細胞들은 紡錘形原始細胞로 되고, 긴 細胞들의 一部가 橫斷分裂을 하여 放射組織原始細胞로 전환된다. 維管束形成層의 特徵은 胚軸의 伸長이 끝나고 약간 뒤에 갖추어지므로, 胚軸에 있어서 前形成層으로 부터 維管束形成層의 轉移過程은 漸進的인 것으로 해석된다.

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(Received August 13, 1988)

Explanation of Figures

Figs. 2–7. Transverse sections of the uppermost region of *Glycine max* hypocotyl (Bar equals 125 μm in Fig. 2 and 31 μm in Figs. 3–7). C=central bundle, L=lateral bundles, P=phloem, PC=procambium, PX=primary xylem, I=interfascicular regions, VC=vascular cambium, SX=secondary xylem.

Figs. 2–3. The stage of early primary growth showing central and lateral bundles in Fig. 2. Fig. 3 is high magnification of central bundle of Fig. 2.

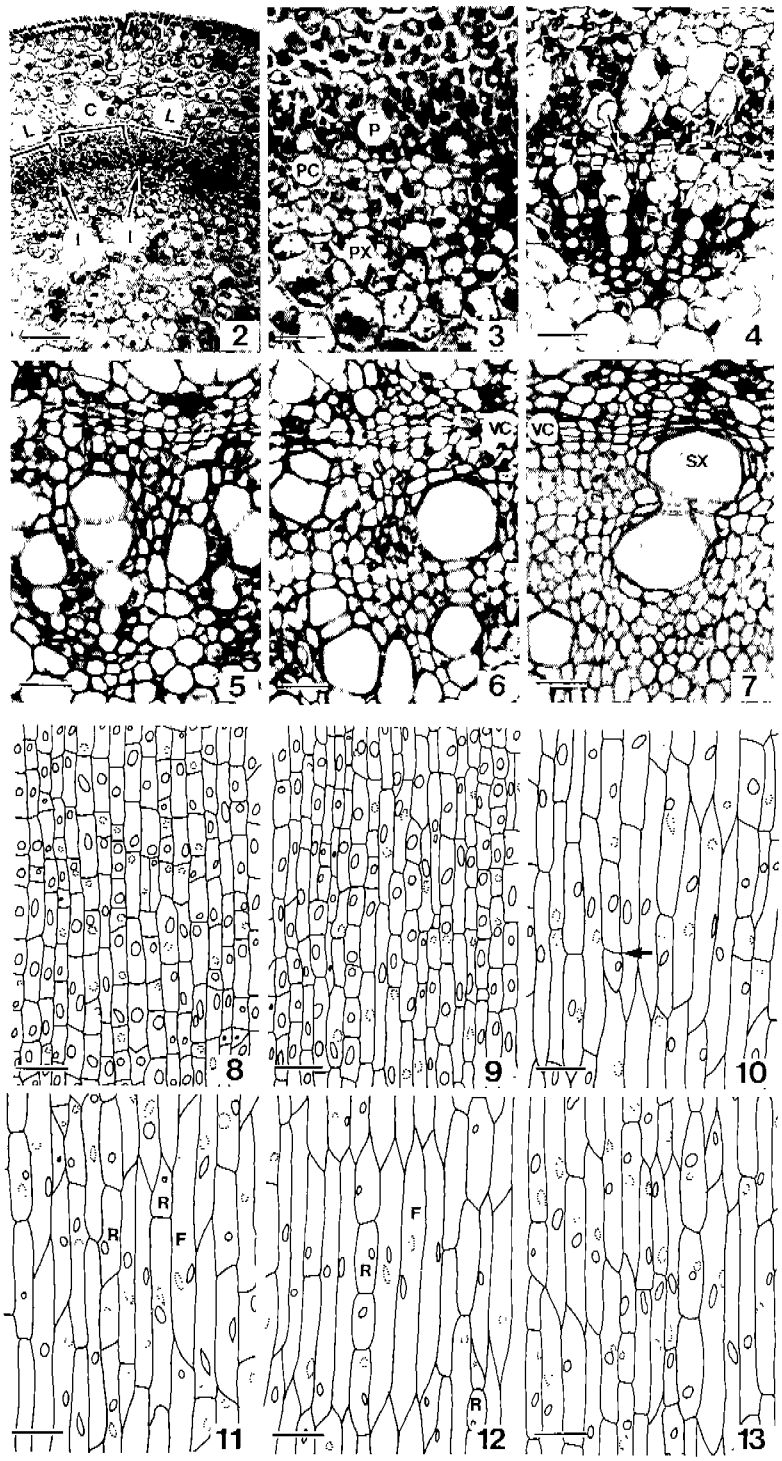
Fig. 4. The stage of later primary growth showing radial division of cells of the vascular meristem (arrows).

Fig. 5. The stage of primary growth completion showing more actively periclinal divisions than in Fig. 4.

Fig. 6. The stage of secondary growth initiation showing differentiation of the vascular cambium.

Fig. 7. Advanced secondary growth showing conspicuous vascular cambium and secondary xylem.

Figs. 8–12. Tangential sections of the vascular meristem of the same stage and region as shown in Figs. 3–7 (Bar equals 31 μm). Fig. 8. Procambium shows homogeneous structure composed of short cells with transverse end walls. Fig. 9. Procambium is essentially homogeneous and some of its cells are elongating. Fig. 10. Vascular meristem shows homogeneous structure composed of elongated long cells with tapering or transverse end walls and transverse division of an elongated long cell (arrow). Fig. 11. Vascular cambium is organized into two systems, elongated fusiform initials (F) and ray initials (R). Fig. 12. Vascular cambium has two distinct systems, almost storied fusiform initials (F) and axial strand of ray initials (R). Fig. 13. Tangential section of segment Ⅳ in 8-day-old seedling hypocotyl of *Glycine max* shows almost the same structure of vascular cambium as in Fig. 11.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