LATERAL THICKENING IN THE STEM OF *AGAVE RIGIDA* MILL. AND *ALOE VERA* L.

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Abstract: The possible methods of lateral thickening in *Aloe vera* L. and *Agave rigida* Mill. are described and illustrated in detail. Primary thickening meristem, vascular cambium and cork cambium all contribute to the thickening of the stem. The vascular cambium is different from that found in dicotyledons and gymnosperms, it produce both vascular tissue and ground tissue (conjunctive tissue) toward the inside and ground parenchyma toward the outside. Some vessel elements are found in macerated secondary tissue of *Aloe vera* L.

INTRODUCTION

Secondary growth occurs commonly in both dicotyledons and gymnosperms. The vascular cambium contributes to the formation of secondary xylem and secondary phloem. In monocotyledons, though secondary growth is known to form in some herbaceous species and many woody plants, a typical secondary thickening is scarce even in the massive arborescent members of Palmae and Pandanaceae. In early studies of secondary growth in monocotyledons there were descriptions of structural variations such as obvious growth rings which were due to variations in lignification and the density and tangential alignment of bundles (Lindinger, 1909a, 1909b; Chamberlain, 1921; Barkley, 1924). Cheadle (1937) mentioned the secondary growth of some Liliflorae as well as in a number of other groups of monocotyledons. Tomlinson and Zimmermann (1969) reported the pronounced eccentric secondary growth in a leaning stem of *Yucca aloifolia*. Recently eccentric secondary growth was described and illustrated in detail in horizontal stems of *Cordyline*, *Yucca* and *Beaucarnea* (=*Nolina*) by J. Fisher (1975).

As described by the workers mentioned above, the derivatives of the vascular cambium (lateral thickening ring) are usually considered to contribute the integral part of the lateral thickening in the monocotyledons. The vascular cambium (lateral thickening ring) is definitely present in *Aloe vera* and *Agave rigida* collected in Taiwan. However as mentioned by some other workers, the activities of some other tissues are also concerned with lateral growth. This study reports the histological changes which are responsible for the increase of the lateral thickening, occurring in the stem of *Aloe* and *Agave*.

MATERIALS AND METHODS

Growing plants of *Aloe vera* L. were obtained from the green house of Botany Department, National Taiwan University, and specimens of *Agave rigida* Mill. were collected in Hengchung (恒春).

Materials from living plants were killed and fixed in formalin-acetic-alcohol (Johansen, 1940). After subsequent washing and dehydration through a tertiary-butanol series, the specimens were embedded in tissuemat. Serial transverse sections were cut at the thickness of 10μ and stained with tannic acid and iron alum with safranin O and orange G (Sharman, 1943).

Some older materials were cut in the transverse plane with a sliding microtome at 20μ.

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After being washed with water and fixed in 50% ethanol, sections were stained with safranin O and fast green (Johansen, 1940). Some fresh materials were macerated with saperoxal to observe the cell types in the secondary tissue.

RESULTS

Lateral thickening by the primary thickening meristem:

In the longitudinal section of the shoot apex of *Agave*, repeated periclinal divisions were observed in the cells near the base of both apical meristem and leaf primordia (Fig. 1B). These divisions resulted in the primary thickening meristem. The primary thickening meristem forms a nearly complete cylinder parallel to the leaf base as well as the periphery of the upper stem which is only interupted by a very small region close to the base of the apical meristem in which no periclinal divisions occur. The smaller the distance from the primary thickening meristem to the apical meristem, the closer the primary thickening meristem is to the leaf base. The cell layers of the primary thickening meristem near the apical meristem are less in number than farther away from it, ranging from 11-37 in the plants of 1 m height. Anticlinal divisions are common in the apex of young stem (Fig. 1C), but the periclinal divisions is more prominent in the apices of older stem.

The initiation of primary thickening meristem in *Aloe* is similar to that in *Agave* except that the primary thickening meristem of the latter is not so closed to the base of the apical meristem and the anticlinal divisions are almost absent (Fig. 1D). The cell layers of the primary thickening meristem of *Aloe* range from 11-13 in the examined materials which are of about 30 cm in height.

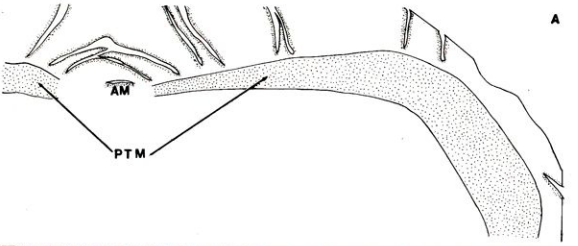
The origin of the vascular cambium:

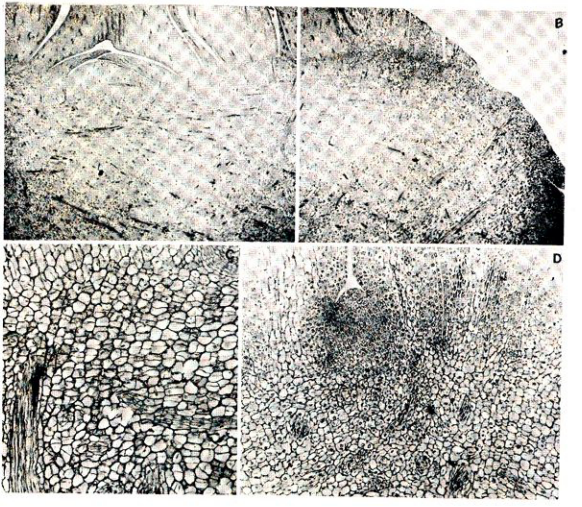
The vascular cambium originates very early in the stems of both *Aloe* and *Agave*. It is situated laterally in the peripheral parenchymatous cortex outside the primary vascular bundles before the complete maturation of the latter. The first sign of the cambial cells is identified by the periclinal divisions at several separated regions of some cortical cells (Fig. 2A). These cambial initials are not arranged in a continuous layer in the early stage. They are more than one cell in thickness in some places after becoming a laterally continuous band. Scott and Brebner (1893) used the term "thickening ring" to designate these peripheral layers of cambial initials. It was found by Röseler (1889) and Schoute (1902) that these cambial initials were at first tiered without a single initial layer being termed an "Etagencambium". In this paper we follow the Fisher's (1975) terminology to use the term "cambial zone" to refer to this meristematic zone.

General description of secondary tissue

The cambial zone precedes a succession of periclinal divisions resulting in radially arranged derivatives. The inner derivatives undergo differentiation to form secondary vascular bundles and parenchymatous conjunctive tissues. The seconday phloem elements mature prior to xylem elements, (Fig. 2C) whereas their outer derivatives give rise to the parenchymatous secondary cortex. Unlike that of dicotyledons and gymnosperms, the secondary vascular bundles of these plants are discrete but radially located in similarly oriented conjunctive parenchyma (Figs. 3B & 5D). Both conjunctive and cortical parenchyma have somewhat thickened walls exhibiting many pits (Fig. 4C). Many raphids can be found in them. (Figs. 4A & 5B).

In macerated materials of both *Aloe* and *Agave* we find a number of very long fiber-tracheids (Fig. 4B, Table 1). The tracheids are always shorter than fiber-tracheids (Table 1). The phloem consists of relatively short sieve tube members with somewhat transverse end walls. The well-perforated vessel elements are common in *Aloe*. All the vessel elements found in the present work have simple perforation plates. But no vessel elements were seen in *Agave*.


A
AM
PTM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C
D

Fig. 1A. drawing depicted from fig. 1B, showing the location of the primary thickening meristem.
Fig. 1B. median longitudinal section of the shoot apex of *Agave*, note the psimary thickening meristem extends along the base of the leaf primordia down to the periphery of upper stem. ×6.9
Fig. 1C. enlarged view of a portion of primary thickening meristem, both anticlinal and periclinal divisions are obvious. ×57
Fig. 1D. median longitudinal section of the shoot apex of *Aloe*, periclinal divistons are prominent in the primary thickening meristem. ×57

Key to labelling; AM, apical meristem; C, cortex; CC, companion cell; CZ, cambial zone; E, epidermis; Pd, phelloderm; Pe, periderm; Pg, phellogen; Ph, phellem; PTM, primary thickening meristem; SE, sieve element; X, xylem.

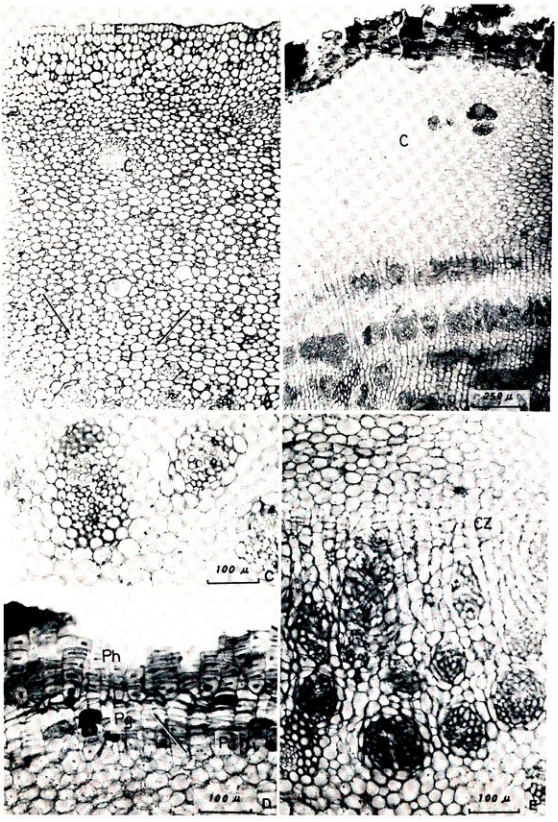
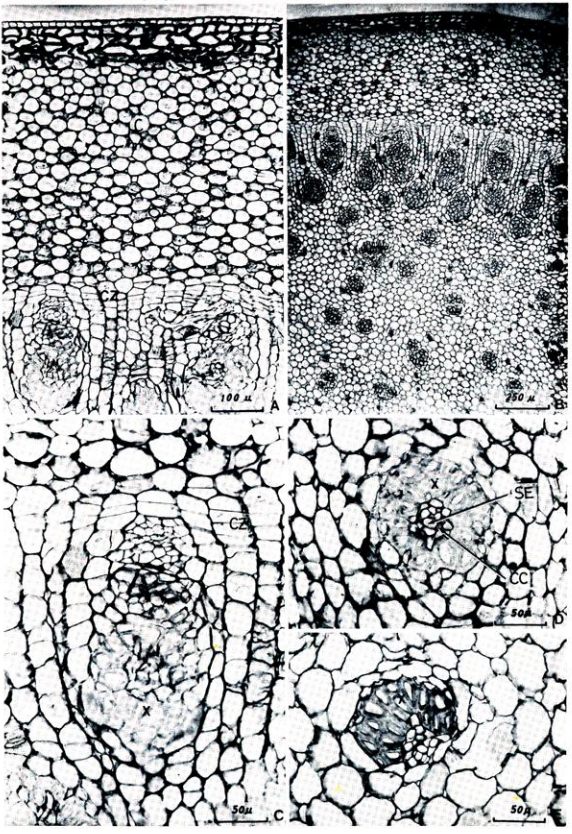

C
250 μ
100 μ
C
Ph
Pe
100 μ
D
CZ
100 μ
E

Fig. 2. Transverse sections of the stem of *Agave rigida* Mill.
A. younger stem, showing the first stage of secondary growth, arrows show the cambial initials.
B. older stem, periderm and secondary vascular bundles have formed.
C. primary vascular bundle with fiber on two sides.
D. higher magnification of Fig. 1B, arrow shows the phellogen. note the cell wall of phelloderm is also thickened.


100 μ
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CC
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D
50μ

Fig. 3. Transverse sections of stem of *Aloe vera* L.
A. showing the periderm under the epidermis, cortex, cambial zone and secondary vascular bundles.
B. showing the arrangement of both primary and secondary vascular bundles.
C. secondary tissue near the cambial zone, note phloem differentiation precede xylem differentiation.
D. the amphivasal secondary vascular bundle, companion cell and sieve tube element are clearly differentiated.
E. the collateral primary vascular bundle.

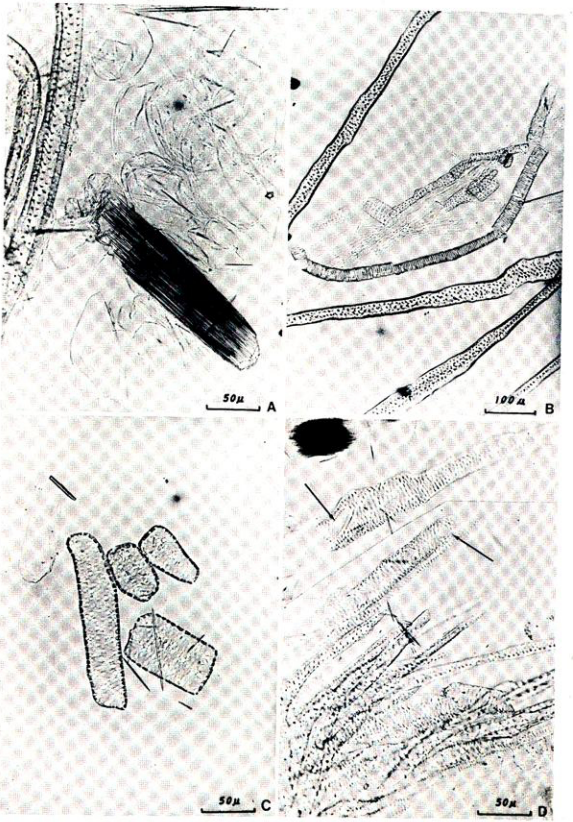
50μ
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Fig. 4. Cell types from the macerated secondary xylem of *Aloe vera* L..
A. parenchyma with raphid crystal
B. tracheid, fiber-tracheid and some secondary parenchyma cell
C. enlarged secondary parenchyma showing the pits on the cell wall.
D. vessel elements with simple perforation plate (arrows).

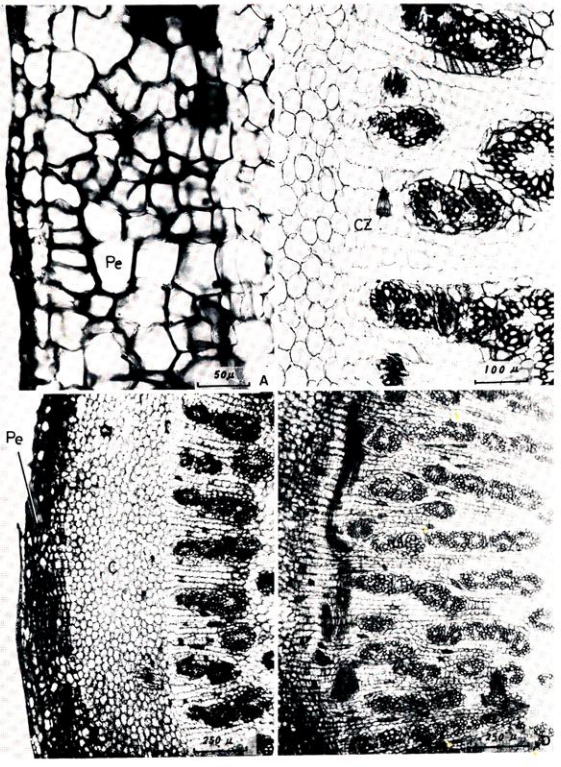
Pe
50μ
A
CZ
100 μ
Pe
C
250 μ
250 μ
D

Fig. 5. Transverse sections of the outer part of older stem of *Aloe vera* L.
A. enlarged periderm, note the phellogen only divide toward the outside and the phellem are irregularly arranged.
B. showing radial arrangement of secondary vascular bundle and the pitted secondary parenchyma, some xylem parenchyma with raphid crystal.
C. showing the thick-walled periderm, cortex, cambial zone and secondary vascular bundle.
D. the secondary vascular bundles anastomose tangentially, some vascular bundles are oriented parallel with the epidermal surface.

Table 1. Size relation between vessel, tracheid and fiber tracheid in μ.

	Vessel element		Tracheid		Fiber-tracheid	
	length	width	length	width	length	width
Aloe	110-125	50-60	1300-1400	25-30	520-2750	25-55
Agave	—	—	1200-1500	15-25	3500-4500	15-30

The secondary vascular bundles of *Aloe* are amphivasal with xylem surrounding a central phloem strand (Fig. 6). Some bundles anastomose extensively in tangential direction (Fig. 5D). The secondary vascular bundles of *Agave* are either amphivasal or collateral (Fig. 7).

The vascular bundles:

The primary vascular bundles are collateral and scattered throughout the section but the secondary vascular bundles are amphivasal in *Aloe* (Figs. 3D & 6) and either collateral or amphivasal in *Agave* (Figs. 2E & 7). They occur in radial files (Figs. 6 & 7).

In transverse section, the primary vascular bundles of *Aloe* are smaller, with a lesser number of xylem and phloem elements than the secondary vascular bundles, but the primary vascular bundles of *Agave* are larger, with a greater number of xylem and phloem elements than the secondary bundles (Table 2).

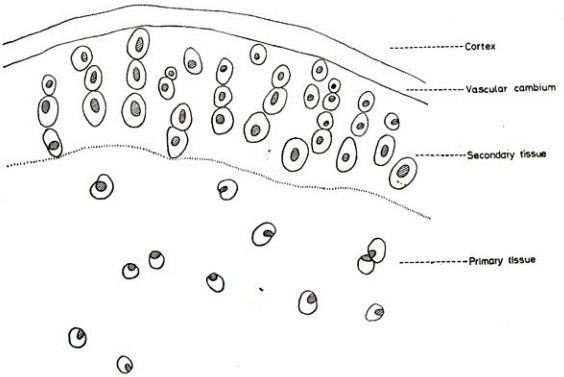

Fig. 6. Drawings showing the arrangement of xylem and phloem in the primary and secondary vascular bundles and the arrangement of primary and secondary tissue in *Aloe vera* L. (striped area indicates phloem)

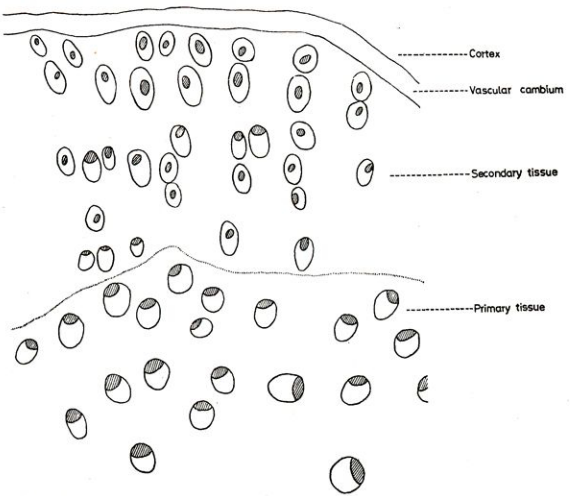

Fig. 7. Drawings showing the arrangement of xylem and phloem in the primary and secondary vascular bundles and the arrangement of primary and secondary tissue in *Agave rigida* Mill. (striped area indicates phloem)

Table 2. The number of cells in the xylem and phloem in primary vascular bundle and secondary vascular bundle of *Aloe* and *Agave*.

plant	primary bundle				secondary bundle			
	cell No. of xylem	average	cell No. of phloem	average	cell No. of xylem	average	cell No. of phloem	average
Aloe	11-29	17	6-15	10.6	14-54	30.8	7-20	13.4
Agave	16-59	37.6	18-57	37	23-42	32.6	15-19	16.4

Periderm formation:

Both *Aloe* and *Agave* produce a periderm somewhat different from that in dicotyledons. In *Aloe*, the phellogen is initiated in the subepidermal layer. It divides periclinally only toward the outside and the products of the divisions undergo a similar pattern of cell division and then lignified to become phellem. Sometimes the phellem cell may divide in various directions so that the phellem is not entirely in a radial file (Figs. 5A & 5C). In *Agave*, the periderm

consists of phellogen, phellem and phelloderm. The cell walls of both the phellem and phelloderm are thickened and lignified in the later stages of development. (Figs. 2B & 2D). The phellogen produce many more cells outward than inward. The phelloderm always consists of only two to three cell layers.

DISCUSSION

The stems of monocotyledons may increase in lateral thickness in three distinct ways. These are: (1) by cell division during the phase of primary growth; (2) by diffuse secondary thickening; and (3) by the action of a type of secondary thickening peculiar to monocotyledons (Philipson, Ward & Butterfield, 1971). *Aloe vera* and *Agave rigida* increase their thickness mainly by the first and third methods. There is a flat zone called the primary thickening meristem below the leaf and sheath primordia. The primary thickening meristem extends along the base of the leaf primordia down to the peripheral part of the upper stem. Fahn (1967) suggested that the activity of this type of meristem is somewhat similar to that of a vascular cambium and if these two types of tissue occur in one and the same plant then the latter develops from the former. But in continuous transverse sections in the stems of *Agave* and *Aloe*, there is a region where neither vascular cambium nor primary thickening meristem can be distinguished so that the cambial zone is not initiated from the primary thickening meristem although in the later stage of development it is possible that they become a continuous structure. Ball (1941) concluded that the primary thickening meristem at first contributes mainly to the thickening of the stem but later is also responsible for the increase in stem height. Both *Aloe* and *Agave* possess a vascular cambium, but its origin and the manner of activity are distinct from that found among dicotyledons. The possible differences between them are listed in Table 3.

Table 3. The possible differences between the development of vascular cambium in monocotyledons and dicotyledons.

	Monocotyledons	Dicotyledons
origin	cortical cells	procambium & ground tissue
initial cell types	rectangular cells or different shapes	fusiform initials & ray initials
derivatives	vascular tissue and conjunctive tissue (ground tissue) toward the inside and parenchyma toward the outside	secondary xylem toward the inside and secondary phloem toward the outside

The monocotyledons rarely form a type of periderm resembling that of the dicotyledons (Philipp, 1923). In *Aloe vera*, the phellem cells are not seriated radially. In *Agave rigida*, both the phelloderm and phellem cells become very thickened and lignified. In *Livistonia*, *Typha*, *Phoenix* and the Gramineae, there may be a modification of the ground parenchyma into a protective tissue by the suberization or thickening and sclerification of the cell walls (Esau, 1965).

Raphids can be found in the ground parenchyma of both primary and secondary tissues. There are more crystals in the secondary tissue than in primary tissue. Cordemoy (1893) worked on the function of the ground parenchyma and noted that this tissue commonly contains calcium oxalate deposited in the form of raphids and other crystalline inclusions, and frequently starch and sometimes oils. In *Aloe* and *Agave*, neither starch nor oil is present in the ground parenchyma.

The secondary tissue produced by the cambial zone toward the inside is easily distinguished from the primary tissue. The primary vascular bundles are scattered irregularly in the ground

parenhyma but the vascular bundles in secondary tissue are radially seriated and embedded in similarly oriented parenchyma which is composed of cells with thickened walls exhibiting many pits. Fisher (1975) commented that this type of parenchyma functions in support.

Fahn (1967) stated that the tracheary elements of the secondary vascular bundles in all monocotyledons that have as yet been studied are all tracheids. But in the macerated secondary tissue of *Aloe*, some well perforated elements have been found. They are shorter than the non-perforated elements and represent simple perforation plates. They are comparable to the typical vessel elements.

Though *Dracaena hookeriana* (Agavaceae) was found to have secondary growth in its roots by cell division in the pericycle (Cheadle, 1937), the secondary thickening of the roots of monocotyledons is not common (Pfeiffer, 1926). In *Aloe vera* and *Agave rigida*, there is no secondary growth in the roots.

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