The Maturation of the Trifoliate Leaf of *Glycine max*

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Introduction

The morphogenesis of the trifoliate leaf of the soybean, *Glycine max*, var. Hawkeye, can be divided into two phases. The first phase is the formation of six layers of histogenic initials and the second is the maturation of these layers into fully developed leaves. As the first phase is still under study, this paper deals with the latter.

Materials and Methods

The material used for sectioning was grown in the greenhouse at Purdue University during the summer of 1959. Collections were made from the first and second trifoliate leaves every other day during their growth from one centimeter in length at the midrib to maturity. Randolph's Modified Navashin Fluid (CRAF) was used as the killing and fixing agent. Following dehydration in ethyl alcohol, the material was embedded in tissuemat, sectioned at 10μ, and stained with chlorazol Black E.

Results

Thirteen days after planting the first trifoliate leaf has developed to a size large enough to handle as a separate unit. At this time the leaflets are still folded and the tissue of the lamina is undifferentiated. A cross section through the lamina of one of the lateral leaflets displays six layers or rows of histogenic initials (Fig. 1).

The two outer layers of cells form the epidermis. The upper layer forms the adaxial epidermis and the lower layer forms the abaxial epidermis. The cells of the epidermis are the first to cease cell division. However, these cells have the longest and greatest period of enlargement (1). At this stage of development these cells are isodiametric. Numerous epidermal hairs are present.

The two layers of cells adjacent to the abaxial epidermis form the spongy mesophyll. With only a few exceptions cell divisions stop in this

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Plate 1.

Figure 1. A cross section of a leaflet of a second trifoliate leaf showing the six layers of the young meristematic cells. The leaflet measured approximately one centimeter long at the midrib. (141X)

Figure 2. A cross section of a leaflet of a first trifoliate leaf exhibiting partial differentiation. The epidermis and spongy mesophyll tissue are expanding while the palisade tissue is still somewhat meristematic. The leaflet measured approximately 2-3 centimeters long at the midrib. (188X)

Figure 3. A cross section of a leaflet of a second trifoliate leaf fully mature. Cell division and expansion have stopped and the intercellular spaces have been formed. The leaflet measured approximately 5-6 centimeters long at the midrib. (188X)

Figure 4. A cross section of the mature petiole of the second trifoliate leaf near the base of the lamina. There is an epidermis, ground parenchyma region, and vascular cylinder present. (150X)

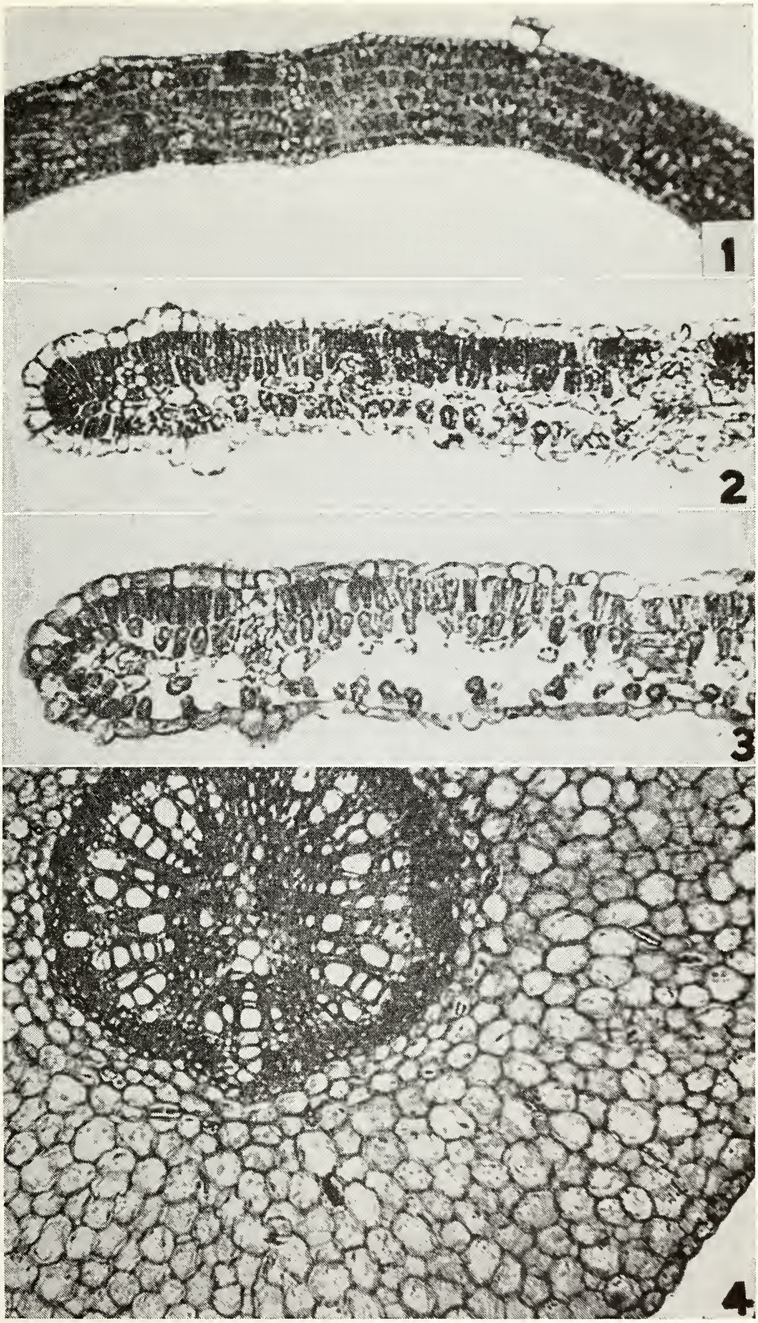

Plate 1

region very soon after the epidermis ceases dividing. Avery (1), in his study on the tobacco leaf, accounts for the large intercellular spaces of the mature spongy mesophyll by the fact that the abaxial epidermis and the spongy mesophyll stop cell division at approximately the same time, and that the epidermis has the greater expansion period. The leaf of the soybean develops in a similar manner.

The remaining two layers form the two layers of palisade tissue. Active cell division continues in this tissue longest. The intercellular spaces are small in contrast to the intercellular spaces in the spongy mesophyll tissue because the palisade cells are still dividing during most of the period of epidermal expansion.

In the intermediate stage of maturation, the upper and lower epidermis and the spongy mesophyll have stopped cell division and begun to enlarge and differentiate (Fig. 2). The palisade layers continue to divide with only a slight amount of differentiation evident. Intercellular spaces are visible in the spongy mesophyll but not in the palisade region.

The abaxial and adaxial epidermal layers form a continuous layer over the margin in the fully mature leaf (Fig. 3). The palisade layer extends around the margin, and has some intercellular spaces. Often the cells of the inner palisade layer are forked while cells of the outer layer remain unforked. The spongy mesophyll layer develops extensive intercellular spaces when its cells reach maximum size. Due to the expansion of the epidermis, the palisade cells with their darkly stained chloroplasts, lose some of the regular arrangement they possessed as undifferentiated cells (Fig. 5). The network arrangement of the spongy mesophyll cells is clearly visible. As previously mentioned, the palisade cells are the last cells of the leaf to cease cell division with only a few exceptions. The vascular endings in the spongy mesophyll layers can be seen in figure 6. Some of the young mesophyll cells remain meristematic and become provascular tissue as the lamina expands (1). The pathway of materials to and from the cells of the lamina can be seen clearly (3).

In the young midrib, a cambium-like region is visible which gives rise to the newly formed xylem and phloem (Fig. 7). Both protophloem and metaphloem are present. There is also some internal phloem present. The crushed protoxylem can be seen along with with the developing metaxylem. Some of the metaxylem cells still contain the cell contents yet show definite secondary wall thickening. In the more fully developed midrib (Fig. 8), the cambium-like region is less distinct due to maturation. The mature xylem and phloem are located in the characteristic arrangement

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Plate 2.

Figure 5. A peridermal section through the palisade tissue of the fully matured leaflet of a second trifoliate leaf. Also shown is the vascular pattern and some spongy mesophyll tissue. The leaflet measured approximately 5-6 centimeters long at the midrib (127X)

Figure 6. A peridermal section through the spongy mesophyll tissue of the fully matured leaflet of a second trifoliate leaf. The vascular endings and their connection to the spongy mesophyll tissue are clearly visable. The leaflet measured approximately 5-6 centimeters long at the midrib. (114X)

Plate 2

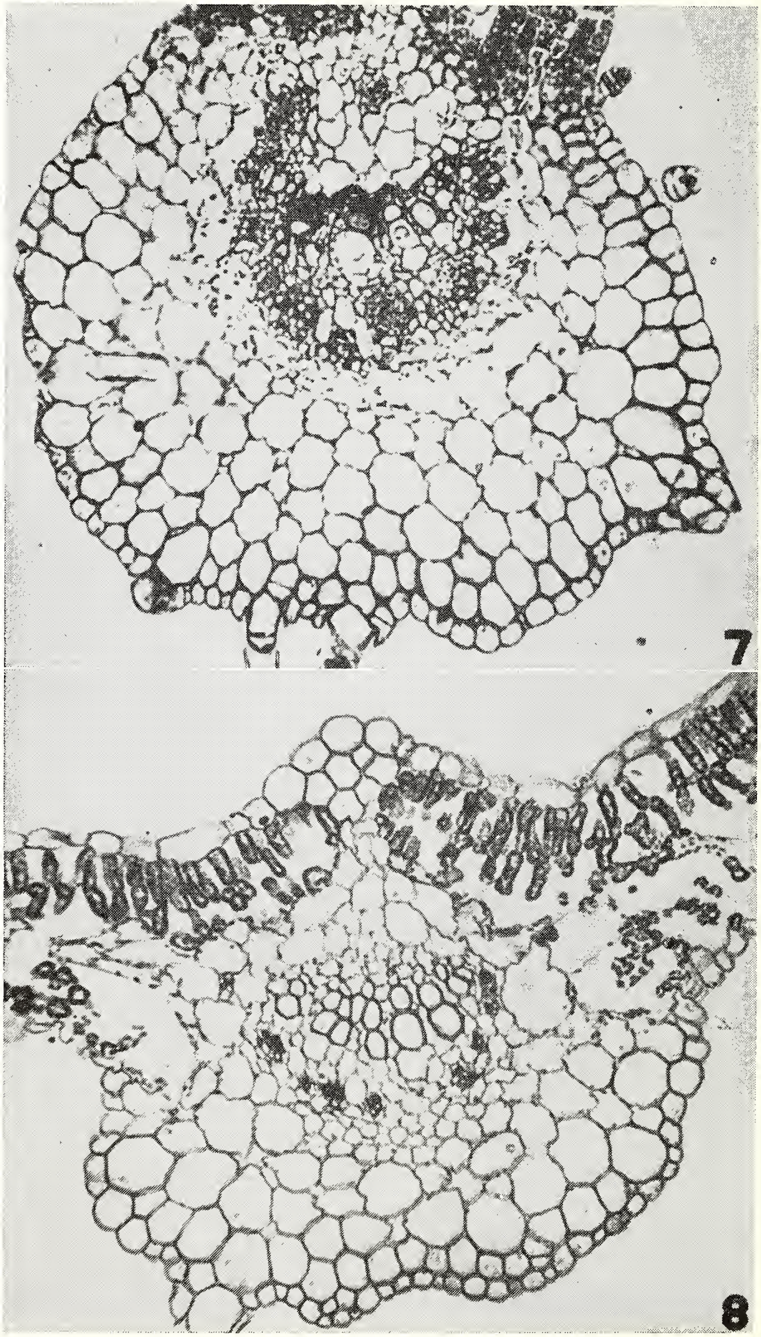

Plate 3

with the phloem occurring in small patches of darkly stained cells on the abaxial side of the xylem.

The epidermis of the mature petiole is pubescent as is the leaf and stem. There is a layer of ground parenchyma (2) or cortical tissue (3) which surrounds the vascular system. The center is occupied by crushed xylem and no pith is present (Fig. 4). Large xylem elements are maturing which suggest the presence of a cambium or cambium-like region. The phloem is oriented towards the periphery of the petiole encircling the xylem (2).

Discussion

Mounts (4) showed one layer of palisade tissue and three layers of mesophyll tissue in *Vitis* and *Catalpa* leaves. The *Malus* leaf is eight to ten layers of cells in thickness; one to three layers being palisade, two forming the epidermis, and the remaining layers forming the mesophyll tissue (3). The tobacco leaf is described as having an upper and lower epidermis, an upper mesophyll which develops into the single palisade layer, a middle mesophyll, and lower mesophyll. The latter two form the spongy mesophyll (1). The soybean is found to possess two outer layers which form the epidermis, two layers which form the palisade tissue, and two layers which form the spongy mesophyll. Avery (1) and MacDaniels and Cowart (3) indicate that whatever the number of layers of cells a leaf primordium may have, this number generally remains constant throughout the life of the leaf. All authors cited and the writers agree that the number of layers of cells of leaf primoidia vary slightly. The sequence of cessation of cell division of the various layers in all the species discussed is essentially the same.

Esau (2), citing the work of Sharman 1942 as an example, indicates that the vascular differentiation of the midrib is acropetal while the small veinlets (Figs. 1, 2, 3) differentiate in a basipetal direction. The vascular development of the soybean follows this pattern.

Once the number of layers of cells of the leaf primordium is established, the soybean leaflets follow the same pattern of differentiation and maturation as that found for other species described by Mounts (4), MacDaniels and Cowart (3), and Avery (1).

Summary

The differentiation and maturation of the trifoliate leaf of *Glycine max* follows a basipetal pattern for the veinlets of the leaf tissue while the midrib is acropetal. Six layers of undifferentiated cells are formed in the lamina. This number is constant throughout the life of the leaf. The two outer layers form the epidermis. The two layers beneath the upper

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Plate 3.

Figure 7. A cross section through the midrib of the young second trifoliate leaf. This shows the epidermis, ground parenchyma, and developing vascular tissue. The leaflet measured approximately one centimeter at the midrib. (229X)

Figure 8. A cross section through the midrib of the mature first trifoliate leaf. Secondary xylem form rows while secondray phloem is collected in small groups. The leaflet measured approximately 5-6 centimeters at the midrib (194X)

epidermis form the palisade tissue and the two layers above the lower epidermis form the spongy mesophyll tissue. The differentiation and maturation pattern for the leaflets of this trifoliate leaf are similar to that of a simple leaf.

Literature Cited

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