DIFFERENTIATION OF VASCULAR TISSUES IN *HIBISCUS SABDARIFFA* LINN.

By B. C. Kundu and S. C. Datta

Botany Department, Presidency College, Calcutta

Received for publication on May 19, 1943

CONTENTS

Introduction

The importance of the fibres of *Hibiscus sabdariffa* L., particularly the variety *altissima*, as a substitute for jute has been stressed by some scientists during the last few years. The present work forms a part of the studies undertaken at the Botanical Laboratory of the Presidency College, Calcutta, on the development and structure of fibres of this plant. This paper deals with only the differentiation of the protoxylem and protophloem; but a detailed anatomy of the plant along with the development, structure and nature of fibres is being continued and the results will be published subsequently.

Material and Methods

The specimens were obtained from plants grown in the Gardens of the Presidency College, Calcutta, from seeds received through the courtesy of the Agricultural Department of the Government of Bengal.

Tips of vegetative shoots were killed in Formalin-Acetic-Alcohol after previous treatment with Carnoy's fluid and taken through grades of alcohol and xylol in the usual way. Sections were cut 8 to 10 μ thick and stained with Safranin and Light Green or Safranin and Fast Green. The cutting of sections was often facilitated by dipping the paraffin blocks in water for 12 to 24 hours.

Observations were also made from materials macerated in 5% chromic acid; the macerated materials were stained with Safranin or aqueous Eosin and mounted in glycerine.

Morphology

The plant is a small woody herb cultivated throughout the hotter parts of India and Ceylon. It completes its life-cycle from the germinating seed to death following fruit production within a single growing season. The stem yields a strong, silky fibre, the *Roselle hemp* of commerce, obtained by retting the stems when the plants are just in flower. It can grow in situations where jute cannot, and that is why it is believed by some that there are immense possibilities for this fibre as a substitute for jute in areas where the latter cannot be cultivated. Besides the fibre, other parts of the plant are also useful. The calyx of the flowers grows along with the fruits and becomes fleshy. It is a valuable antiscorbutic and is often eaten in the form of chutneys and jelleys. The seeds are reported to yield a kind of oil.

The plants attain a height of 8 to 10 feet and sometimes even 15 feet. The leaves are arranged spirally, the phyllotaxy varying from 2/5th in young shoots to 3/8th in vigorously growing shoots. The axillary buds produce branches which do not grow vigorously and as a result the general appearance of the plant is tall and erect.

Shoot Apex

A series of microtome sections of an actively growing stem tip across the vegetative apical bud of a vigorously growing shoot showed 8 leaves crowded around the shoot apex, which are distinctly arranged in a 3/8th phyllotaxy (Fig. 1). There is no indication of the presence of an axillary bud in the axils of the first four primordia. An axillary bud develops in the axil of the 5th primordium. The development of the first internode is initiated between the 4th and 5th primordia.

The general manner of distribution and increase in the size of the leaf primordia in the shoot apex is given below in a tabulated series :—

Primordium	Level of insertion below the shoot apex*	Depth of insertion†	Length of free limb
	μ	μ	μ
1	10	20	50
2	20	30	290
3	60	60	770
4	140	130	1890
5	240	130	..
6	460	130	..
7	660	240	..
8	1060	..	..
9	1860	..	..

* Level of insertion is the point where the leaf primordium is becoming free from the stem.

† The region between the level of insertion of the primordium and the point where it is completely fused with the axis has been termed the "depth of insertion".

Vascular Differentiation

Differentiation of vascular tissues is first noticed in the median bundle of the 3rd primordium which may be attributed to its more vigorous growth in comparison with that of primordium 2. The length of the free limb of primordium 3 was observed to be 770 μ while primordium 2 was only 290 μ in length. For this vigorous growth a greater food supply was required and as a result differentiation of vascular elements was found to take place in order to meet this demand. One protoxylem and one protophloem element was observed in this primordium (Figs. 1 and 2); the former differentiates as an isolated element at 120 μ below apex. From here the course of the xylem was traced in the free limb of this primordium and it was found to extend to a distance of 540 μ in the leaf while the phloem element continued still further up to a distance of 600 μ in this primordium. The lateral traces are also differentiated in primordium 3 but they remain in the procambial condition. The position of the protoxylem element is almost opposite to the sieve tube element of the protophloem which is also produced in the same desmogen strand.

The simultaneous differentiation of protoxylem and protophloem is contrary to the usual development of phloem before xylem, but it is not entirely unknown [see Kundu (1942) on jute].

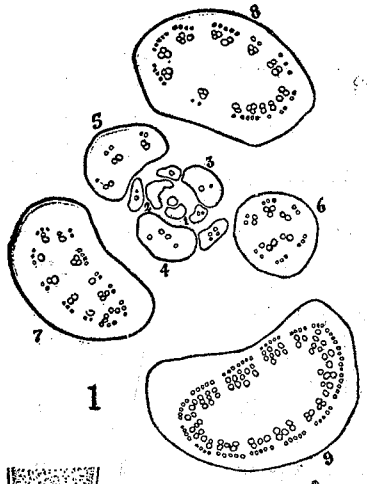
At the insertion of primordium 3 there is no distinct vacuolation of the axis, but a continuous prodesmogen strand is found to have been developed. Vacuolation of the axis commences 100 μ below the growing tip, where only a few cells in the central region are found to have been vacuolated. The process of vacuolation is very rapid and only 60 μ below this point (*i.e.*, 160 μ below the growing tip) the central region (pith) is found to have been completely vacuolated. The process of vacuolation in cortical cells also begins simultaneously with those of the central region and at the level of 180 μ below the growing tip the central as well as the cortical regions are found to have been highly vacuolated.

At the insertion of primordium 4 most of the cells in the central region are found to have been vacuolated. At the point of fusion of primordium 4 with the axis, it is found that vacuolation has started even in the ray cells giving rise to the formation of 4 strands.

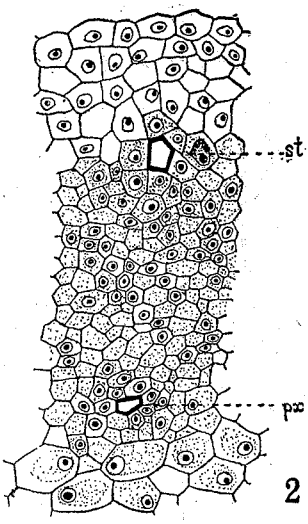
The primary rays are uniseriate and the procambial cells are in more or less radial alignment resembling those of jute (Kundu, 1942).

Protoxylem

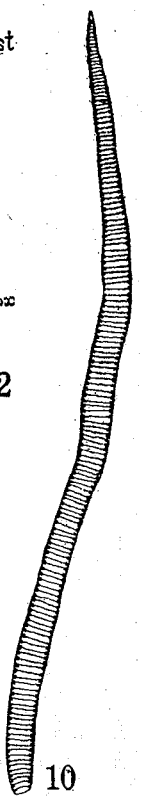
The first xylem elements to appear are the small uninucleate spiral elements. In the procambial strand 120 μ below the apex, the first indication of protoxylem vessel was noticed. A few of the cells of the procambium were found to increase markedly in length and were seen to be associated with surrounding meristematic cells, which divide radially longitudinally and irregularly



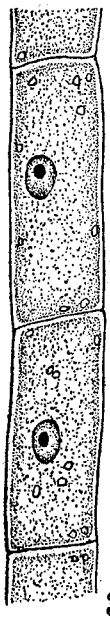
1



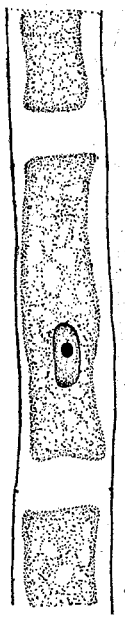
2



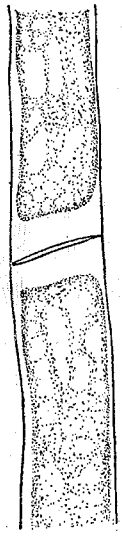
10



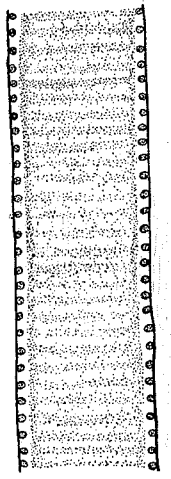
3



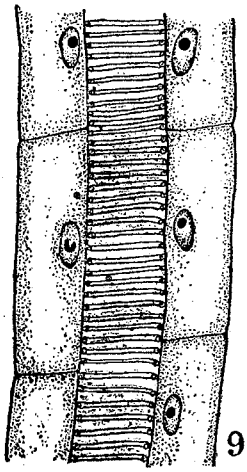
4



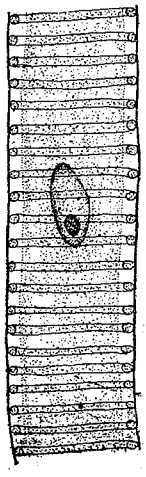
5



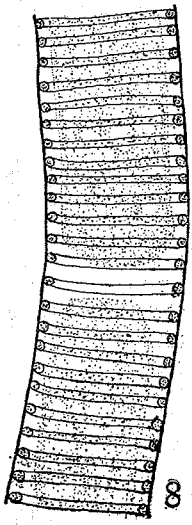
6



9



7



8

Text-figs. 1–10.—Fig. 1. Transverse section of the apex of a vigorously growing shoot (×40). Fig. 2. Transverse section of a portion of the 3rd primordium showing differentiation of a sieve tube and a protoxylem vessel. *st.*, sieve tube; *px.*, a protoxylem vessel (×467). Fig. 3. Vessel mother cells in longitudinal section (×650). Fig. 4. File of protoplasts (×650). Fig. 5. Vessel segment with pectin film (×650). Fig. 6. Portion of a vessel segment showing deposition of bases and banding of cytoplasm. In macerated material and also in sections from fixed material the protoplasm appears to be contracted from the cell wall (×650). Fig. 7. Portion of a vessel segment with spiral secondary thickening and protoplasmic contents (×650). Fig. 8. Portion of a vessel segment with spiral thickening and degenerating cytoplasm (×650). Fig. 9. Vessel segment showing spatial adjustment of surrounding cells (×300). Fig. 10. An entire tracheid-like vessel segment at one end of which there is a perforation; the other end is pointed and has no opening (×367).

to give rise to xylem parenchyma cells. These cells are narrower than the vessel segments and are distinguished from the neighbouring pith cells by their size and protoplasmic contents. The developing vessel segments are in vertical series and they develop directly from the desmogen cells without further division.

A young vessel mother-cell is a growing meristematic cell full of contents (Fig. 3). A few such cells are arranged end to end; during the earlier stages the end walls of such cells are clearly visible separating the young vessel elements. At the first stage of vessel differentiation the transverse end walls of the vessel mother-cells are dissolved and the protoplasm is seen to contract from the transverse and longitudinal walls. Though the end walls become dissolved the protoplasts of the developing vessel segments retain their individual characteristic and they form what is called the "file of protoplasts" (Fig. 4) (Priestley, Scott and Malins, 1935). This "file of protoplasts" is formed at a very early stage in vessel differentiation when the elements are extending and expanding and its existence is for a very short period. Soon after the formation of the file of protoplasts, a film of pectin is found to be developed at the position of the end wall in the form of a membrane (Fig. 5). In a longitudinal section this "pectin film" appears to be more or less lenticular in shape and limited above and below by two dark lines enclosing a light coloured substance. It disappears at a later stage, when the vessel segment has attained its maximum extension. Sometimes it exists even when deposition of thickening matters on longitudinal walls has started. While disappearing, the pectin films first appear as faint threads; this thinning down of the pectin film indicates that it will soon be dissolved.

The first indication of secondary thickening is noticed in the deposition of bases on the inside walls of the vessel segments, when they have expanded fully (Fig. 6). These bases are thickenings on primary wall of vessel segments and they form localised projections into the cavity of vessel segments. On these projections thickening matters are laid. The presence of these projections on the wall of the vessel segment prevents the thickening matters to come into close contact with the wall. The bases appear as

continuous lines running along the centre of the thickening bands. They are not perceptible in highly lignified bands.

The protoplasm in the vessel segment suffers certain changes during the formation of the vessel. In a young cell it is uniform and dense, but as the cell increases in volume, the protoplasm contracts from the longitudinal walls as well as from the transverse end walls and fine vacuoles appear in it. Later on, with the formation of file of protoplasts these small vacuoles coalesce to form large vacuoles. In *Hibiscus* the banding of cytoplasm in finely and coarsely vacuolated regions at an early stage of vessel differentiation has not been observed as found by Barkley (1927) in *Trichosanthes* at a very high magnification. Nevertheless, when bases are deposited along the entire wall of the vessel segment the protoplast appears to be banded into thick and thin regions (Fig. 6). During all the stages of vessel differentiation and deposition of secondary thickenings the protoplast persists and the nucleus occupies central position of a vessel segment (Fig. 7). The nucleus degenerates shortly after the deposition of cellulose spiral band, but the cytoplasm persists upto the earlier stages of lignification in a fully developed vessel (Fig. 8).

It has been stated that protoplast appears to be banded after the deposition of bases. Along the thickened regions of the protoplast, deposits are formed on the walls over the bases and gradually they thicken and appear as rods. Staining reactions show that these bands are composed of pecto-cellulose but later on they change their chemical nature and become lignified.

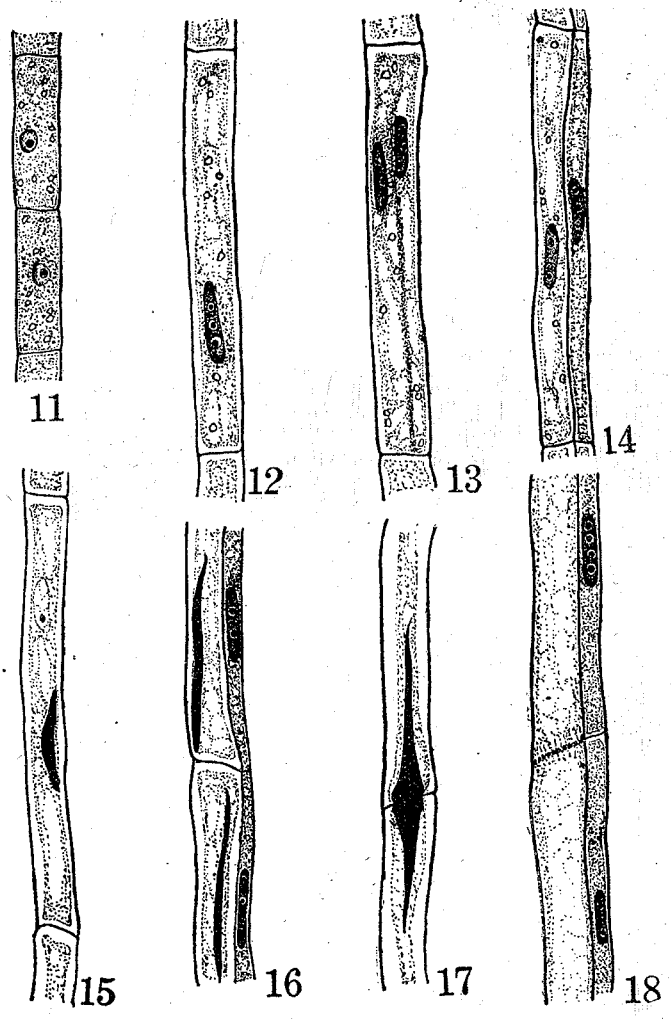
The vascular elements of the protoxylem region sometimes have pointed end walls and as a result they appear somewhat like tracheids. In Fig. 10 is shown a vessel segment at one end of which there is perforation, the other end is pointed and has no opening.

Spatial adjustment.—During the differentiation of the vessels, the vessel segments exert great pressure on the surrounding cells which are mostly meristematic. As a result, these cells undergo rapid divisions radially longitudinally, transversely or sometimes irregularly. Thus, when the vessel segment is elongating, spatial adjustment is brought about by a rapid division of the surrounding parenchyma cells (Fig. 9).

Protophloem

The ontogeny of primary sieve tubes in *Hibiscus* has been studied from transverse as well as from longitudinal sections. The development of the protophloem strand begins with the differentiation of the first sieve tube at the outer periphery of the median desmogen strand of primordium 3 (Fig. 2). In transverse sections a protophloem sieve tube can be recognised by its swollen wall which stains markedly with Light Green or Fast Green. If this element is followed downwards it is found that it soon becomes crushed and degenerated. Thus if we follow from the apex we find that a protophloem element develops from a procambial cell

and this element becomes crushed downwards due to the continued pull and pressure exerted by surrounding tissues. Hence evidently, primary sieve tubes begin to arise acropetally from those formed earlier, while the older ones are continually crushed and become obliterated.



Text-figs. 11–18.—Fig. 11. Phloem mother-cells (×975). Fig. 12. An enlarging phloem mother-cell (×975). Fig. 13. Stage showing the cutting of a companion cell (×975). Fig. 14. A young sieve tube segment with a companion cell (×975). Fig. 15. Sieve tube segment with slime body and degenerating nucleus (×975). Fig. 16. Sieve tube segments with elongated slime bodies (×975). Fig. 17. Sieve tube segments with slime plug (×975). Fig. 18. A mature sieve tube with a companion cell (×975).

The sieve tubes have one companion cell each. Phloem parenchyma consists of somewhat elongated cells with transverse walls. The companion cells are not easily recognised in transverse sections in very young regions because many of the procambium cells are similar in size to the sieve tubes and companion cells and have as dense protoplasts as the companion cells.

The development of a sieve tube from its earlier stages can best be followed from a study of longitudinal sections as well as from macerated material. A young protophloem element (a sieve tube mother-cell) is an elongated cell with heavy stained nucleus, uniform cytoplasm, and transverse end walls (Fig. 11). The nucleus contains one or more nucleoli. There are also several other small spherical particles in the mass of the protoplast and they are the characteristic sieve tube plastids.

In the next stage the sieve tube mother-cell is found to extend and the nucleus also becomes elongated and 2 or 3 nucleoli are found to be present in it. The cytoplasm also becomes less dense and vacuolated (Fig. 12).

Later on the nucleus divides with the formation of two nuclei of the same size and a wall is formed dividing the cell longitudinally into two each containing a single nucleus. One of these cells forms the sieve tube element and the other the companion cell. The young sieve tube is larger than the companion cell. The companion cells can also be differentiated from the sieve tubes by their dense protoplasts and prominent nuclei (Figs. 13 and 14).

After the companion cell has been cut off the cytoplasm of the sieve tube element becomes highly vacuolated; its nucleus loses its chromaticity, swells up and degenerates gradually. At the time the nucleus degenerates, one or more characteristic slime bodies appear in the mass of cytoplasm (Fig. 15). In the early stage they are spindle-shaped, but later on tape like slime bodies have been found in the sieve tube element (Fig. 16). The slime bodies of two adjoining sieve tube elements usually come in contact with the wall separating the two elements and form what are called the "slime plugs" (Fig. 17).

When slime plugs are formed the protoplast of the sieve tube elements contract from the longitudinal walls which become thickened. This thickening of the walls is first noticed at the stage when slime bodies appear after the cutting off of companion cell and it reaches its maximum prominence in the latest stage of development of the element. The thickened walls appear glistening and pearly in transverse sections and have been termed "nacree" walls (Leger, 1897) (Fig. 2).

After the walls have thickened, the slime plug degenerates, the end wall develops very fine perforations and the cytoplasm forms a very thin parietal layer with network of very fine strands traversing the lumen.

Discussion

Literature on the differentiation of vessels in angiosperms is scanty and the descriptions somewhat incomplete and contradictory. It is well known that the differentiation of vascular tissues is intimately connected with the development of leaves and the first differentiated vascular elements of the stem appear in localised regions which constitute the leaf traces.

In dicotyledons the differentiation of vessels usually commences in the primordium at its level of insertion on the axis and from there progresses downwards into the stem and upwards towards the apex of the primordium. This has been observed by a number of observers (Trecul, 1881, 1891 ; Weiss, 1883 ; De Bary, 1884 ; Priestley and Swingle, 1929 ; and other recent workers) and is now regarded as well established. Our observation is also in agreement with the above fact.

Regarding the relative time of appearance of the first phloem and first xylem elements, Sanio (1863) reported that in stems phloem elements appear before xylem. Russow (1872) and Leger (1897) also found the appearance of phloem before xylem ; they considered it to be a normal phenomenon in the differentiation of vascular elements in Phanerogams and Cryptogams. Chang (1935) reported that the development of the vascular tissue in a primordium begins with the differentiation of a sieve tube. But in *Hibiscus* it is found that protoxylem and protophloem elements differentiate at the same time and this condition is in agreement with the observations of Kundu (1942) on jute.

According to most authors the cells destined to form the protoxylem vessels are uninucleate but F. M. Scott (1937) asserts that in *Ricinus* they are uninucleate in the younger internodes but multinucleate in the older ones. In *Hibiscus* there is no such coenocytic stage and the nucleus occupies a central or more or less central position in the vessel segment until its disintegration at the maturity of the vessel.

Regarding the breaking down of the end wall there is a great difference of opinion. According to Eames and MacDaniels (1925) the vessel segments reach their full size and permanent shape with the end walls unperforated. Esau (1936) found that in Celery the end walls break down after the maturation of the secondary wall. Barkley (1927) also refers to the late breaking down of end walls. F. M. Scott (1937) on the other hand observed that the end walls disappear in the coenocytic stage. According to Priestley and his co-workers (1935, 1938) the end walls of a vessel segment disappear at a very early stage even before the segments have attained their maximum extension. Sometimes the transverse wall splits in the middle, contracts and sticks to the sides of the vessel segments thus forming what is called the *rim*. Though the wall dissolves the protoplasts of different vessel segments do not come in contact but retain their individual characters and form what is known as " file of protoplasts ". Our observations on *Hibiscus*

agree with those of Priestley, Scott and Malins (1935) and Majumdar (1940) and the "file of protoplasts" develops at a very early stage of the differentiation of the vessels. The pectin film, which becomes deposited in the position of the dissolved end wall soon after the formation of the file of protoplasts appears lenticular in shape and looks like a thickened end wall as described by Esau (1936.)

Regarding the manner of disappearance of the end walls Esau regards it as a process of dissolution. Eames and MacDaniels also describe it similarly and in *Robinia* suggested an association of the nucleus with the formation of the vessel pores. In *Hibiscus* the end walls of the developing vessel segments disappear very early, long before they attain their maximum extention. The manner of disappearance of the end walls could not be followed thoroughly. It must be an abnormally rapid process as in none of the prepared slides intermediate stages of the breaking up or the dissolution of the end walls could be traced. But it seems to us to be a process of dissolution; in no case, however, was the nucleus found to be associated with the disappearance of end walls, as its position remains unchanged and it lies at or near the centre of vessel segment until its disintegration at the maturity of the vessel. In *Hibiscus* the pectin film which is formed later and resembles the end wall described by Esau (1936), does not dissolve before the maturation of the secondary wall; sometimes, however, it disappears before the deposition of thickening matters. The nucleus does not take any part in the disappearance of the pectin film. The part played by the pectin film in the development of vessels is not quite clear.

The early stages of secondary thickening and the progress of lignification takes place while the protoplast still persists. Barkley (1927) has pointed out that before secondary thickening the protoplast is banded into alternate finely and coarsely vacuolated regions. In *Hibiscus* banding of cytoplasm has been observed simultaneously with deposition of bases. The deposition of bases before an actual secondary thickening of the vessels seems to be a special feature. This phenomenon was first noticed by Rothert (1899) (cited in Haberlandt, 1914). Eames and MacDaniels (1925), Barkley (1927), Esau (1936) and Scott (1937) did not mention anything about the deposition of bases. Recently Majumdar (1940) has observed them. The bases are formed in earlier stages and appear in the pattern of secondary thickenings on the walls. On these bases cellulose bands are deposited. The bands later on become lignified, and the protoplast of the vessel segment persists till lignification is complete.

As regards the manner of spatial adjustment during differentiation of vessels, Eames and MacDaniels state that no cells are broken during adjustment. Krabbe (1886) noticed tearing apart of adjacent cells during development of vessels and this was confirmed by Priestley, Scott and Malins (1935) in the expansion

of secondary xylem vessels. Esau (1936) has also pointed to the same method of adjustment. In *Hibiscus* spatial adjustment is brought about by active radial, longitudinal, transverse or irregular division of the surrounding cells and this agrees with the observations of Majumdar (1940) in *Heracleum*.

The failure of some workers (Eames and MacDaniels, 1925) to recognise sieve tubes in the protophloem may perhaps be attributed to their comparatively indistinct sieve plates. Nevertheless, existence of sieve tubes in the protophloem was reported by many early workers like Russow (1872) (originator of the term protophloem), De Bary (1884), Lesage (1891), Leger (1895, 1897) and Chauveaud (1897, 1900) and more recently by Chang (1935) and Esau (1934, 1935, 1936, 1938).

As regards the course of development of the protophloem, Baranetzky (1900) held that the protophloem differentiation went on simultaneously over the entire extent of the leaf trace. Griffiths and Malins (1930) have, however, observed that the protophloem elements differentiate in continuity with the phloem of the older parts of the plant, and that the first sieve tube of a particular leaf trace proceeds acropetally from the stem towards the leaf. These views have been confirmed by Chang (1935), Esau (1938) and Priestley and Scott (1938). The observations of the present writers are also in agreement with these.

The appearance of slime bodies during the development of the sieve tube has been recorded by several workers. Crafts (1933, 1934) reported that in tobacco they occur only in the primary sieve tubes. These slime bodies are generally characteristic of plants belonging to a certain family. The slime bodies in Solanaceæ have been found to be of the same nature by Kotila and Coons (1923), Doolittle and McKinney (1923), Kofoid and others (1923), Artschwager (1924), and Crafts (1933, 1934). Slime drops have been observed in the Cucurbitaceæ by Wilhelm (1880), Fischer (1886), Le Comte (1889) and Crafts (1932); and spindle-shaped slime bodies have been observed in Leguminosæ by Strasburger (1891), Baccarini (1892), Staritz (1893), Doolittle and McKinney (1923) and Bailey (1923). Some plants show no constancy of shape of the slime bodies, as in *Vitis* (Wilhelm, 1880). In *Hibiscus*, slime bodies were found to be spindle-shaped and occasionally tape-like in structure.

The nature of the slime bodies is proteinaceous and it is in agreement with the observations of several workers. Fischer (1885, 1886) reported that slime bodies become dissolved in the sieve tube sap in mature elements and according to Le Comte (1889) they disappear from the sieve tube cytoplasm by passing into the vacuole making the contents more viscous. The disappearance of slime bodies generally agrees in time with disintegration of nucleus but our observation shows that they persist even after the degeneration of the nucleus as found in the Leguminosæ by Strasburger (1890).

The majority of workers agree that the mature sieve tube does not possess any nucleus and that the sieve tube nucleus disappears as a discrete body (Wilhelm, 1880), Janczewski (1881, 1882), Schmidt (1882), Russow (1882), Strasburger (1882, 1887, 1891), Artschwager (1924), Crafts (1932, 1933, 1934), Esau (1934, 1935, 1936, 1938). Certain others report that they have seen nuclei even in the mature sieve tubes of some plants (Fischer, 1886; Le Comte, 1889; Schmidt, 1917). In *Hibiscus*, the nucleus has been found to disappear at an early stage when the slime bodies are appearing.

The sieve tube leucoplasts can be seen during the first stages of specialization of a sieve tube (Briosi, 1873; Wilhelm, 1880; Fischer, 1886; Crafts, 1934; Esau, 1934, 1935, 1936) and this corresponds with our observation on *Hibiscus*. The leucoplasts arise in the cytoplasm of young sieve tubes but may enter the vacuole in mature elements (Fischer, 1886; Crafts, 1933, 1934) and they are sometimes retained by the sieve tube until the elements begin to collapse.

Summary

1. Differentiation of protoxylem and protophloem elements takes place simultaneously in the median desmogen strand of the third leaf primordium. Vacuolation of the axial cells starts 100 μ below the growing tip and 60 μ further below it is found to be completely vacuolated.

2. The first protoxylem elements which differentiate from the desmogen are small rectangular cells (vessel mother-cells). During the first stage of vessel differentiation the longitudinal walls of the enlarging vessel mother-cells remain thin but the transverse walls disappear. The protoplasts of contiguous vessel segments do not mix but retain their individual characteristics and form "file of protoplasts". After formation of the file of protoplasts, a "pectin film" appears in the region of the transverse wall in the form of a membrane and separates the two developing vessel segments. This film which appears like an end wall is dissolved at a later stage in the development of the vessel.

3. During the process of secondary thickening of vessel segments, bases are laid down on their longitudinal walls in the pattern of the thickening matter to be deposited; on these bases cellulose bands are deposited and these bands later become lignified.

4. The protophloem element (the sieve tube mother-cell) is an elongated cell having dense protoplasm and a number of spherical granules (the sieve tube plastids). This cell rapidly extends and by its longitudinal division a companion cell is cut off. Afterwards the nucleus of the sieve tube element gradually degenerates and one or more spindle-shaped or tape-like slime bodies appear in it. At this time the longitudinal wall of the sieve tube becomes thickened and the end walls develop fine perforations. The slime bodies degenerate and the cytoplasm forms a thin

parietal layer with network of very fine strands traversing the lumen.

LITERATURE CITED

Artschwager, E. F. (1918) "Anatomy of the potato plant with special reference to the ontogeny of the vascular system," *Jour. Agr. Res.*, **14**, 221–52.

——— (1924) .. "Studies on potato tuber," *ibid.*, **27**, 809–35.

Baccarini, P. (1892) .. "Intorno ad una particolarita dei vasi cribrosi nelle Papilionaceæ," *Malpighia*, **6**, 53–57 (cited by Esau, 1938).

Bailey, I. W. (1920) .. "The cambium and its derivative tissues.—III. A reconnaisance of cytological phenomenon in the cambium," *Amer. Jour. Bot.*, **7**, 417–34.

——— (1923) .. "Slime bodies of *Robinia Pseudo-Acacia* L.," *Phytopath.*, **13**, 332–33.

Baranetzky, J. (1900) .. "Recherches sur les faisceaux bicollatéraux," *Ann. Sci. Nat. Bot.*, **12**, 261–332.

Barkley, Grace (1927) .. "Differentiation of vascular bundles in *Trichosanthes anguina*," *Bot. Gaz.*, **83**.

Briosi, Giovanni (1873) .. "Ueber allgemeines Vorkommen von Stärke in den Siebröhren," *Bot. Zeitung*, **31**, 306–14, 332–34, 338–44.

Chang, C. Y. (1935) .. "Differentiation of protophloem in angiosperm shoot apex," *New Phyt.*, **34**, 21–29.

Chaveaud, G. (1897) .. "Sur l'evolution des tubes criblés primaires," *Compt. Rend. Acad. Sci.*, **125**, 546–47.

——— (1897) .. "Recherches sur le mode de formation des tubes criblés dans les racines des Dicotyledons," *Ann. Sci. Nat. Bot.*, **12**, 333–94.

——— (1897) .. "L'appareil conducteur des plantes vaculaires et les phases principales de son evolution," *ibid.*, **13**, 113–438.

Crafts, Alden S. (1932) .. "Phloem anatomy, exudation and transport of organic nutrients in cucurbits," *Plant Physiology*, **7**, 183–225.

——— (1933) .. "Sieve tube structure and translocation in the potato," *ibid.*, **8**, 81–104.

——— (1934) .. "Phloem anatomy in two species of *Nicotiana*, etc.," *Bot. Gaz.*, **95**, 592–608.

Doolittle, S. P., and McKinney, H. H. (1923) "Intracellular bodies in the phloem of certain plants and their bearing on the mosaic problem," *Phytopath.*, **13**, 326–29.

Eames, A. J., and MacDaniels, L. H. (1925) *An Introduction to Plant Anatomy*, New York.

Esau, Katherine (1934) .. "Ontogeny of phloem in the sugar beet (*Beta vulgaris* L.)," *Amer. Jour. Bot.*, **21**, 632–44.

——— (1936) .. "Ontogeny and structure of collenchyma and vascular tissues in celery petioles," *Hilgardia*, **10**, 431–67.

——— (1936) .. "Vessel development in celery (*Apium graveolens* L.)," *ibid.*, **10**, 479–84.

——— (1938) .. "Ontogeny and structure of the phloem of tobacco," *ibid.*, **11**, 343–406.

Esau, Katherine (1939) .. "Development and structure of the phloem tissue," *Bot. Rev.*, **5**, 373–432.

Fischer, Alfred (1886) .. "Neue Beitrage zur Kenntnis der Siebrohren," *Ber Verhandl. Konigl. Sachsisch. Gessel Wissensch. Leipzig Math-Phys.*, **38**, 291–336 (cited by Esau, 1938).

Griffiths, A. M., and Malins, M. E. (1930) "The unit of shoot growth in dicotyledons," *Proc. Leeds Phil. Soc. Sci.*, **2**, 125–39.

Haberlandt, G. (1914) .. *Physiological Plant Anatomy*. Eng. Trans.

Hill, A. W. (1908) .. "The histology of the sieve tubes of angiosperms," *Ann. Bot.*, **22**, 245–90.

Janczewski, E. De (1881) .. "Etudes comparées sur les tubes cribreux," *Mem. Soc. Nationale Sci. Nat. et Math. Cherbourg*, **23**, 209–350 (cited by Esau, 1938).

Kofoid, C. A., Severin, H. H. P., and Sweezy, O. (1923) "Nelson's spiral bodies in tomato mosaic not protozoa," *Phytopath.*, **13**, 330–31.

Kotila, J. E., and Coons, G. H. (1923) "Trypanosome-like bodies in Solanaceous plants," *ibid.*, **13**, 324–25.

Krabbe, G. (1886) .. *Das Gleitende Wachstum bei der Gewebebildung der Gefasspflanzen*, Berlin.

Kundu, B. C. (1942) .. "The anatomy of two Indian fibre plants, *Cannabis* and *Corchorus* with special reference to fibre distribution and development," *Jour. Ind. Bot. Soc.*, **21**, 93–128.

Le Comte, H. (1889) .. "Contribution a l'etude du liber des Angiospermes," *Ann. Sci. Nat. Bot.*, **10**, 193–324.

Leger, L. J. (1897) .. "Sur la différenciation et le développement des éléments libériens," *Comp. Rend. Acad. Sci.*, **125**, 619–20.

——— (1897) .. "Recherches sur l'origine et les transformations des éléments libériens," *Mem. Soc. Linn. Normandie*, **19**, 49–182.

Lesage, Pierre (1891) .. "Sur la différenciation du liber dans la racine," *Comp. Rend. Acad. Sci.*, **112**, 444–46.

Majumdar, G. P. (1940) .. "Development and structure of protoxylem vessels in *Heracleum Sphondyllium*," *Proc. Leeds Phil. Soc. Sci.*, **3**, 642–51.

Priestley, J. H., and Swingle, C. F. (1929) "Vegetative propagation from the standpoint of plant anatomy," *U.S. Dept. Agr. Tech. Bul.*, **151**, 1–98.

———, Scott, L. I., and Malins, E. M. (1935) "Vessel development in angiosperms," *Proc. Leeds Phil. Soc. Sci.*, **3**, 42–54.

———, and Scott, L. I. (1936) "Vascular anatomy of *Helianthus annuus* L.," *ibid.*, **3**, 159–73.

Russow, E. (1872) .. "Vergleichende Untersuchungen betreffend die Histologie (Histographie und Histogenie) der vegetativen und sporenbildenden Organe und die Entwickelung dur Sporen der Leitbundel-Kryptogamen, mit Berucksichtigung der Histologie der Phanerogamen, ausgehend von der Betrachtung der Marsiliaceen," *Mem. Acad. Imp. Sci. St. Petersbourg*, Ser. 7, **19**, 1–207 (cited by Esau, 1938).

——— (1882) .. "Sur la structure et développement des tubes cribreux," *Ann. Sci. Nat. Bot.*, **14**, 167–215.

Sanio, Carl (1863) .. "Vergleichende Untersuchungen über die Zusammensetzung des Holzkörpers," *Bot. Zeitung*, **21**, 357–63, 369–75, 377–85, 389–99, 401–12.

Schimdt, W. (1917) .. *Bau und Funktion der Siebröhre der Angiospermen*, Jena.

Scott, F. M. (1937) .. "Differentiation of spiral vessels in *Ricinus communis*," *Bot. Gaz.*, **99**, 69.

Staritz, C. (1893) .. "Ueber einen neuen Inhaltskorper der Siebröhren einiger Leguminosen," *Fetschrift z. 250 jahriger Jubelfeier d. Gymnasiums z. St. Maria Magdalena, Breslau* (cited by Esau, 1938).

Strasburger, E. (1882) .. *Ueber den Bau und das Wachstun der Zellhäute*, Jena.

———— (1887) .. *Das botanische Practicum*, Jena.

———— (1891) .. *Ueber den Bau und die Verrichtungen der Leitungsbahnen in den Pflanzen, Histologische Beiträge*, **3**, Jena.

Thoday, D. (1922) .. "On the organisation and differentiation in the stem of the sunflower," *Ann. Bot.*, **36**, 489–510.

Trecul, A. (1881) .. "Recherches sur l'ordre d'apparition des premiers vaisseaux dans les organs aériens," *Ann. Sci. Nat. Bot.*, **12**, 251–381.

Weiss, J. E. (1883) .. "Das markständige Gefässbundelsystem einiger Dikotyledonen in seiner Beziehung zu den Blattspuren," *Bot. Centbl.*, **15**, 280–95, 318–27, 358–67, 390–97, 401–15.

Wilhelm, K. (1880) .. *Beiträge zur Kenntnis des Siebröhrenapparates dicotyler Pflanzen*, Leipzig (cited by Esau, 1938).