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PHLOEM DIFFERENTIATION IN *PHASEOLUS MUNGO*

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Abstract

Immature protophloem sieve elements present in the embryo of *Phaseolus mungo* mature at about the time of germination. The sieve elements differentiate both acropetally and basipetally. There are numerous protophloem sieve tubes in the upper hypocotyl. These are connected by anastomosis to four sieve tubes, in the root. The protophloem sieve tubes are accompanied by hyperchromatic phloem parenchyma. Hyperchromaticity is the greatest near the differentiating end in the root. Vascular bundles in the cotyledon are amphicribal. Thin deposits of callose are present in some sieve plates but absent in others.

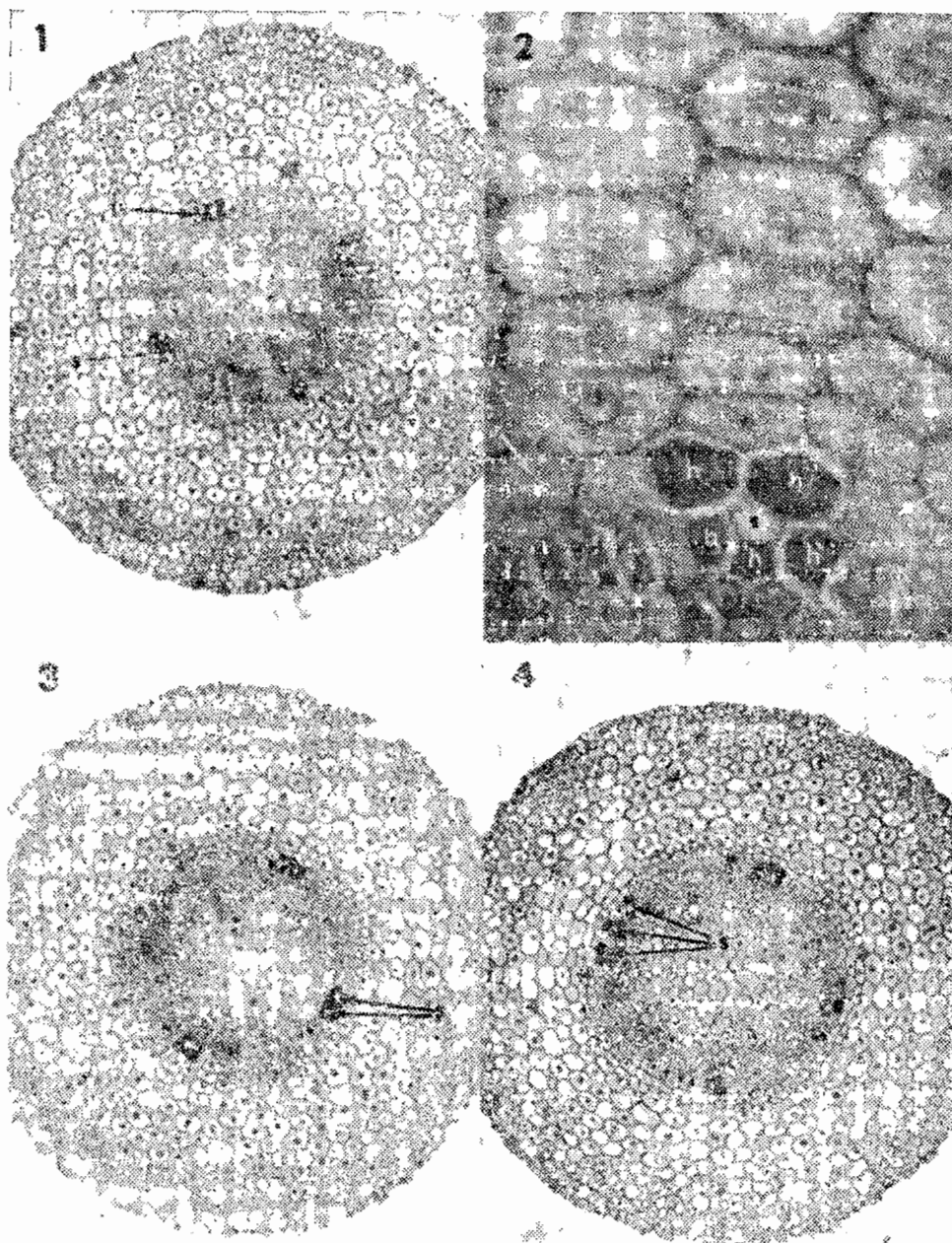
Introduction

Considerable attention has been given to the ontogeny of the secondary sieve element but little is known about the differentiation of the primary sieve element. The longitudinal course of protophloem differentiation, in particular, is poorly understood. The available literature shows that the initial course of the protophloem sieve element has been studied only in a few plants (Esau, 1965; Haque & Isa, 1977; Miller & Wetmore, 1945; Moens, 1963). The protophloem may differentiate either acropetally or basipetally or in both ways depending upon the location of its first appearance (Ealfour, 1957; Bisiaputra, 1961; Hayat & Cawight, 1965; Evert *et al.*, 1969). In *Phlox* the protophloem differentiates bidirectionally into the distal part of the cotyledon and towards the hypocotyl root axis (Miller & Wetmore, 1945). At the cotyledonary node it splits and extends into the epicotyl. According to Compton (1912) all strands of protophloem are cotyledonary in most legumes.

As the immature protophloem sieve elements had been found in the hypocotyl of the mature embryo (Haque & Engelman, 1977), the present work was undertaken to investigate and report the differentiation of the protophloem sieve element in the soaked seeds and young seedlings of *Phaseolus mungo*.

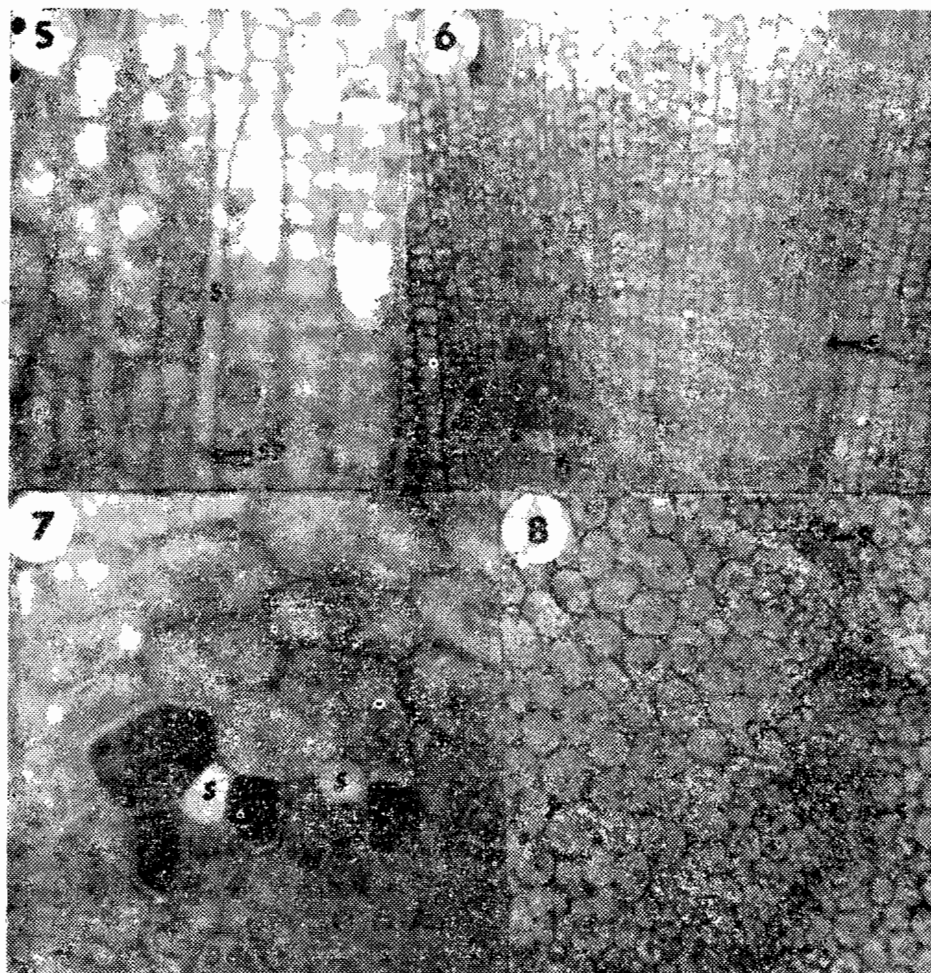
Material and Methods

The experiment was conducted in the Department of Biology, Texas A & M University, U.S.A. with the soaked seeds and young seedlings of *Phaseolus mungo* L. The seeds were obtained through the courtesy of Mr. J.E. Engelman. Woodward



- Fig. 1. Transverse section of a 3 hr old seedling showing 4 protophloem poles in the root. Each pole has one sieve tube(s) encircled by hyperchromatic phloem parenchyma (h). X 85.
- Fig. 2. Higher magnification of a sieve tube(s) than those in Fig. 1. The single-layered pericycle (at the arrow) including some of the hyperchromatic cells (h). X 750.
- Fig. 3. Transverse section through the upper part of the root in a 3 hr old seedling showing 1, or 2 closely associated sieve tubes(s) in each pole. X 85.
- Fig. 4. Transverse section of the lower hypocotyl of a 3 hr old seedling showing 3 sieve tubes(s) in each pole. They are more widely separated than in Fig. 3. X 85.

protophloem sieve tubes lack companion cells. In *Phaseolus mungo* no companion cells but hyperchromatic phloem parenchyma was detected in the hypocotyl root axis (Fig. 2,3,7). This finding coincides with the report of Haque & Isa (1977) for



- Fig. 5. Longitudinal section of the axis of the embryo at about the point of germination (12 hr after soaking) showing protophloem sieve tube(s) with slime plugs (SP). X 1000.
- Fig. 6. Longitudinal section of the root of a 6 hr old seedling showing a row of protophloem sieve tubes(s), accompanied by hyperchromatic cells. X 240.
- Fig. 7. Transverse section of the lower part of the hypocotyl of a 6 hr. old seedling showing two sieve tubes (s). Each sieve tube is encircled by a group of hyperchromatic phloem parenchyma. X 640.
- Fig. 8. Transverse section through the hypocotyl (just below the node) of a 3 hr old seedling showing the distribution of protophloem sieve tubes (s) in ares. No sieve element is present outside the pole of coprotoxylem (CP). X 160.

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Corchorus. Resch (1961) found such hyperchromatic phloem parenchyma along with sieve tubes. He, however, has reported that a small fraction of the sieve elements possess companion cells in the roots of *Lepidium*, *Sinapis* and *Cucurbita* but he did not find companion cells in the roots of *Rhoeo* and *Tradescantia*. Without showing any evidence Douth (1932) has, however, reported the presence of companion cells along with the protophloem sieve tubes in the root of *P. vulgaris*. Within 6 hours after germination three-fourths of the root is traversed by the mature protophloem sieve tubes. The sieve tubes are long in the hypocotyl (Fig. 6).

In the hypocotyl root axis of young seedlings, rows of tannin cells are found within and just inside the primary phloem. These are large and conspicuous cells in the hypocotyl and root. Compton (1912) and Douth (1932) have also reported "tannin sacs" in the stem and hypocotyl but not in the root of *Phaseolus* and other legumes.

Thin deposits of callose on some sieve plates were detected in both paraffin and hand sections. Other sieve plates lacked detectable callose. After the seedlings were immersed in water at 45°C for 15 minutes (Currier, 1957) a considerable amount of callose was found in the protophloem sieve tubes of the hypocotyl root axis and the cotyledons. These results add to the examples of sieve elements that seem to normally lack callose (Currier, 1957; Evert & Derr, 1964). But the results do not lead to a conclusion as to whether the small amounts of callose detected were a result of wound reaction (Engleman, 1965).

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for 9-10 hours, show immature protophloem sieve elements in the petiole and at the base of the cotyledon. The state of maturity of these elements is similar to that of the hypocotyl of the dry seed. The immature sieve elements at the base of the cotyledon proceed adaxially along with the major procambial strands as seen in the longitudinal sections of the cotyledon of the seed at about the point of germination (10-12 hours of soaking). Almost simultaneously some of the minor strands of the procambia are traversed by a few immature sieve elements. The maturation of sieve elements in the cotyledon proceeds from the base. It is very rapid particularly in the larger procambial strands. Most of the sieve elements of both major and minor procambial strands of the cotyledon become mature in the 18 hours old seedling. Some immature sieve elements are, however, found in a few minor procambial strands of the 24 and 30 hours old seedlings. These possibly do not attain maturity and get withered along with the cotyledon. This finding is in agreement with that of Haque & Isa (1977) for *Corchorus*. Ultimately the protophloem and metaphloem sieve elements are established in the entire outer periphery of the procambial strands of the cotyledon as seen in the transverse sections of the procambia. In the 18 hours old seedlings xylem appears in the phloem ring of the cotyledon. The arrangement of vascular tissue in the cotyledon of *P. mungo* is amphicribal. Compton (1912) has reported collateral arrangement of vascular tissue in the cotyledons of *P. hemandesii*. According to Doult (1932), an amphicribal vascular bundle is located in the pulvinus of the simple leaf in *P. vulgaris*. The sieve tubes of the cotyledon possess companion cells and are smaller in diameter than those of the axis.

In the hypocotyl of the seeds, soaked for 8-11 hours, the chromaticity of the nuclei and nucleoli at the immature sieve elements gradually decreases with an increase in vacuolation. The cell wall as well as the length and diameter of the sieve element increases considerably. Highly disorganised nucleus has been observed in some cases as seen in the plastic sections of the hypocotyl of the seeds soaked for 11 hours. The protophloem sieve elements in the hypocotyl root axis of the seed at about the point of germination (12 hr after soaking) seem to be empty except for slime plugs (Fig. 5). These are fully mature protophloem sieve elements. Haque & Isa (1977) have also found mature sieve elements in the hypocotyl of the sprouting seed of *Corchorus*. The protophloem sieve elements of the hypocotyl differentiate acropetally into the cotyledon and basipetally into the root. Such bidirectional differentiation of protophloem has been reported for various plants (Balfour, 1957; Bisalputra, 1961; Hayat & Canright, 1965; Miller & Wetmore, 1945). Unger (1952) reported that protophloem differentiates acropetally and basipetally from the base of the cotyledon of *Echinacea*. In *Phlox* the protophloem differentiates bidirectionally into the distal part of the cotyledon and towards the hypocotyl root axis (Miller & Wetmore, 1945). The direction of differentiation of protophloem depends upon where it appears first.

There are numerous protophloem sieve tubes in the upper hypocotyl (Fig. 8). These are connected by anastomosing to four sieve tubes in the root (Fig. 1.3.4). In the lower hypocotyl, where the number of sieve tubes is greater than four, each pole may have 2-4 adjacent sieve tubes as seen in cross sections (Fig. 4.7). Anatomosis of protophloem sieve elements has occurred in the lower part of the hypocotyl root axis of *Phaseolus mungo* (Fig. 3.4,7,8). Anastomosis of protophloem has been reported for various plants (Haque & Isa, 1977; Swift & O'Brien, 1970; Thompson & Heimsch, 1964). Anastomosis may be total or partial. Temporary anastomosis has also been reported in the scutellum of wheat (Swift & O'Brien, 1970).

In the root, the protophloem sieve tubes are accompanied by hyperchromatic phloem parenchyma (Fig. 2.7,8). Hyperchromaticity is the greatest near the differentiating end in the root. In the root no companion cells were detected. In general

Oklahoma, U.S.A. The mature embryo consisted of a thin epicotyl, two fleshy cotyledons and a stout hypocotyl root axis. The cotyledons were oblong and cordate at the base. The seeds were germinated on moist filter paper in 10-12 hours at room temperature (25°C) under conditions of ordinary laboratory illumination. Herbarium specimens of seeds and seedlings were deposited in the Texas A & M University Tracy Herbarium.

The seeds, soaked for 3, 6, 9, 10, 11 and 12 hours, and the seedlings, 3, 6, 9, 12, 18, 24, 30, 42 and 60 hours old, were fixed in FAA and craft III and dehydrated through a tertiary butyl alcohol series (Sass, 1958). Sections, 6-12 micron thick, were stained in 0.05% (W/V) safranin (in 50% alcohol) and 0.003% (W/V) fast green (in equal parts of xylene and ethanol) after an overnight exposure to 0.5% (W/V) tannic acid (in 50% alcohol). Small pieces of vascular bundles were embedded in plastic and sectioned at 0.5 micron according to the standard method of electron microscopy (Engleman, 1965; Haque & Engleman, 1977). These thin sections were stained with toluidine blue for observations with light microscope. Seedlings, 1, 2, 3 and 4 days old, were fixed in acetic alcohol (1:3) and cleared, using papain and NaOH as suggested by Rodin & Davis (1967). The cleared materials were observed in ordinary light or in crossed polarizers to study the longitudinal course of phloem differentiation.

Flourescence microscopy was used to investigate callose in protophloem sieve elements, using water-soluble aniline blue (Currier, 1957). To detect callose the following steps were undertaken: paraffin sections were obtained from the whole hypocotyl root axis and pieces of cotyledon fixed in craft III within about a minute after cutting. Freehand sections were also made from fresh roots and cotyledons. The fresh sections were immediately placed in aniline blue solution containing 10-50% glycerol.

Results and Discussion

Phloem is the first vascular tissue formed in *Phaseolus mungo*. It matures at about the time of germination. Xylem does not appear until the seedlings are 18 hours old. The vascular pattern of the seedling is similar to that of *P. vulgaris* (Doutt, 1932). The root is tetraarch (Fig. 1,3). One pole parallels the axis under each cotyledon, and two poles are intercotyledonary. Similar observations have been reported for *Phaseolus* and other legumes (Compton, 1912; Dodel, 1872; Doutt, 1932; Haris *et al.*, 1921). Pith appears in the upper part of the root. Transition is of an intermediate type occurring mostly within the hypocotyl which coincides with the reports of Dodel (1872), Freeman (1969), Gerard (1881), Perez & Roland (1963), and Van Tieghem (1871). Sieve tubes ramify in the hypocotyl. Vascular bundles in the cotyledon are amphicribal.

In the course of germination the partially developed protophloem sieve elements in the hypocotyl of the mature seed (Haque & Engleman 1977) advance towards maturation as seen in the transverse and longitudinal sections of the soaked seeds. Seeds soaked for 3 hours show no apparent change in the development of the sieve element. Considerable shrinkage of the protoplasm with conspicuous vacuolation has been observed in the seeds soaked for 6 hours. Repeated trial showed similar phenomenon. The nuclei and the nucleoli are found to be intact as seen in the thin plastic sections. The walls of these sieve elements are, however, thicker than those of the dry seed. The shrinkage of the protoplasm and the thickening in the cell wall of the sieve element accelerate along with the time of soaking. Seeds, soaked